CONTENTS

An Investigation to Determine the Sensitizing Agent in Cattle Tested with Mammalian and with Avian Tuberculins—William H. Feldman and Harold E. Moses..... 3

The Nerve Supply to the Bovine Mammary Gland—L. E. St. Clair..... 10

Intestinal Pathology in Experimental Bovine Esophagostomiasis—John S. Andrews and Jose F. Maldonado..... 17

Observations on the Pathology of Dairy Calves on Low Vitamin A Diets—W. T. S. Thorp, H. A. Keener, S. I. Bechdel and N. B. Guerrant..... 27

A Method of Determining the Reproductive Efficiency of Cattle—H. E. Kingman and H. E. Kingman, Jr..... 32

Studies on Equine Encephalomyelitis. II. A Nonantigenic Strain of Pigeon-Brain Virus—Robert Graham and Norman D. Levine..... 42

Equine Periodic Ophthalmia—T. C. Jones..... 45

Control of the Hog-Kidney Worm (Stephanurus Dentatus)—W. L. Threlkeld and E. P. Johnson..... 72

An Experiment to Determine the Effect of the Growth Hormone on the Anterior Lobe of the Pituitary Gland of Swine—D. D. Giles..... 77

Further Studies on Overeating (Enterotoxemia) of Feedlot Lambs—G. S. Harshfield, Floyd Cross and Alvin B. Hoerlein..... 86

The Bacterial Content of Goat Milk—C. S. Bryan..... 92

Observations on Bacterin Treatment of Salmonella Typhi-Murium Infections in Chinchillas—Don R. Coburn, William H. Armstrong and Psyche W. Wetmore..... 96

The Effects of Hyperplastic Endometritis on the Blood Cytology of the Dog—M. L. Morris, James B. Allison and John I. White..... 100

Inheritance as a Factor in Poultry-Disease Research—C. A. Brandly and Nelson F. Waters. 105

A Study of Neoplastic Disease in a Flock of Chickens—Carl Olson, Jr..... 111

The Experimental Use of Organic Sulfur Compounds for the Prevention of Cecal Coccidiosis in Chickens—C. A. Herrick, C. E. Holmes and D. L. Degiusti..... 117

Organization of the Research Council, American Veterinary Medical Association..... 128

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An Investigation to Determine the Sensitizing Agent in Cattle Tested with Mammalian and with Avian Tuberculins*

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ON NOVEMBER 1, 1940, the amount of tuberculous infection among the cattle of the United States was considered officially to be less than 0.5 per cent.[1] This accomplishment, which required 23 years of effort and the expenditure of approximately $275,000,000, must be considered one of man's most outstanding achievements in the control of an infectious disease. That the incidence of bovine tuberculosis has been reduced to such a satisfactory minimum is indeed evidence of the sound wisdom of the methods followed.

One should be mindful, however, of the fact that the disease has been diminished rather than eliminated. Adequate foci of perpetuation of the disease still exist, as evidenced by the fact that during the fiscal year ending June 30, 1940, 8,384 cattle, exclusive of positive reactors to the tuberculin test, that were slaughtered under federal supervision, showed lesions of tuberculosis. Of this group the disease in approximately 2,000 was sufficiently severe to require condemnation or sterilization of the carcass. Although the total number of tuberculous animals slaughtered was not large, the potential danger of such animals is obvious and provides reason for insisting that there be no lessening of the vigilance necessary to maintain the gains achieved. As long as there remains undetected a single tuberculous bovine animal, the possibility for the transmission of the disease to healthy animals must be considered a threat to gains that have occurred during the first 23 years of our national bovine tuberculosis-eradication program. If the successful efforts of the past can be maintained and the present trend continues, it is reasonable to expect that bovine tuberculosis, which was once a relatively prevalent disease, will become eventually a rarity.

As the program of eradication has continued, not only have the frankly tuberculous animals been eliminated, but in addition the retests have resulted in detecting the infection in its incipient stage. As a consequence, it should follow naturally that the percentage of cattle which react positively to tuberculin will diminish and that the percentage of positive reactors without confirmatory lesions of tuberculosis will increase.

Not only is the problem created by the so-called nonvisible-lesion reactors disconcerting, but also it incites in the minds of some a reasonable doubt as to whether or not tuberculin is a diagnostic substance of absolute specificity. At the present time there exists no entirely satisfactory explanation of this problem. We are aware of the prevalent ideas that have been advanced.* Many of the explanations are entirely tenable and perhaps even likely, but difficult to support by an adequate amount of positive evidence. Although we do not wish to weaken confidence in tuberculin as a product of tremendous and proved

*From the Mayo Foundation.

[1]Wight, H. S.: The general live stock tuberculosis situation. Proc. 44th Ann. Meet. U. S. Live Stock San. Assn. (Chicago, 1940), pp. 121-125.

*For an analytical consideration of the so-called nonvisible problem the contribution by Hagan[2] may be consulted.

[2]Hagan, W. A.: The no-lesion case problem in the tuberculosis eradication campaign. Cornell Vet., xxi (1931), pp. 163-176.

value in detecting infections by the tubercle bacillus, it is, nevertheless, important that we embrace a realistic concept of the "non-visible-lesion" situation and honestly admit that at the present time there is no convincing explanation for the large majority of the cattle that react to tuberculin but that are without definite lesions of tuberculosis at the time of slaughter. The implications of the problem are many. Unless the true explanation is established eventually, it is conceivable that cattle may continue to react to tuberculin long after infection with tubercle bacilli has ceased to occur.

To obtain additional data on this problem an investigation, which was suggested in 1939 by Dr. C. H. Fauks,* was carried out with his coöperation. The objectives were as follows: (1) information concerning the sensitivity to avian and to mammalian tuberculin of cattle from a territory where tuberculosis of poultry is commonly present and (2) determination of the type or types of tubercle bacilli presumably responsible for sensitivity to avian and to mammalian tuberculin in the cattle that were tested.

MATERIAL AND METHODS

The material had its origin from cattle within the state of South Dakota and represented 101 tuberculin reactors from 99 separate herds comprising a total of 3,073 cattle. All of the material was obtained from animals whose first reaction to mammalian tuberculin alone had been recorded as suspicious or indefinite. Sixty days after the first test the "suspects" were retested with avian and mammalian tuberculin prepared in the laboratories of the Bureau of Animal Industry. The tuberculins were injected intracutaneously. Both caudal folds and both sides of the vulva were utilized as sites for the injection of the tuberculins. The avian tuberculin was injected into one caudal fold and into the corresponding side of the vulva and the mammalian tuberculin was injected into the other caudal fold and the corresponding side of the vulva. The results were recorded after 72 hours and the animals classified either as reactors or as negative. The results of the tuberculin tests were recorded in the standard code designed for this purpose.

Animals that reacted positively to the tuberculin test were slaughtered under the supervision of the federal meat-inspection service. At the time of necropsy, tissues were selected for subsequent laboratory examination.

Unless lesions were found elsewhere, the routine procedure was to secure for laboratory examination one or more mesenteric lymph nodes and the retropharyngeal lymph nodes. When received at the laboratory, a small portion of each tissue was preserved for subsequent histologic examination and the rest of the material was emulsified for the making of cultures and for the inoculation of guinea pigs. Summaries of the findings at necropsy of the 101 animals compiled from the postmortem records show that lesions that were considered to be tuberculous were present in 44, or approximately 43.6 per cent, of the carcasses; so-called skin lesions only were found in 17, or 16.8 per cent, while in 40, or approximately 39.6 per cent, no lesions of tuberculosis were observed.

Since there is a possibility that sensitivity to tuberculin in cattle may occur as a consequence of avian tubercle bacilli, the presence or absence of tuberculosis among the farm poultry of each of the 99 farms was determined by veterinarians in the employ of the Bureau of Animal Industry. It was established that avian tuberculosis was present on 23 of the premises.

Cultures.—After treatment with oxalic acid to eliminate other than acid-fast bacteria, each specimen was used to inoculate four slants of egg-yolk-agar medium and four slants of glycerinated (3 per cent) egg-yolk-agar medium. The slants were incubated for ten weeks before being discarded as negative. Positive cultures of acid-fast bacilli were transplanted and kept for subsequent typing by tests of pathogenicity.

Animal Inoculations.—Each of the specimens obtained from the respective cattle was used to inoculate two guinea pigs. All injections were made subcutaneously. The animals were kept under observation for eight weeks and then were killed for necropsy. Tissues showing tuberculous-like lesions at the time of necropsy were cultured routinely for tubercle bacilli.

Typing.—All strains of tubercle bacilli obtained were examined for physical differences characteristic of the avian, bovine and human varieties of *Mycobacterium tuberculosis*. All of these strains were essentially similar and were considered characteristic of the bovine type of the organism. One strain for each of the cases yielding acid-fast bacilli was used to inoculate two additional guinea pigs and two rabbits. From the results of these tests for pathogenicity it was possible to identify the respective strains with a reasonable degree of certainty.

*Inspector in charge for the U. S. Bureau of Animal Industry at Pierre, S. Dak., in 1939. Since Dr. Fauks' transfer to another station, the work has been carried on under the supervision of Dr. Neal Plank.

TABLE I—Summary of Material Examined for Tubercle Bacilli from 101 Cattle That Reacted to Tuberculin

Lesions at Necropsy	Animals	Tubercle Bacilli Demonstrated			
		Culture Mediums		Guinea-Pigs	Positive Cases
		Glycerinated	Nonglycerinated		
Tuberculosis	44	2 cases*	19 cases	23	23
"Skin lesions" only	17	None	None	None	None
None found	40	None	None	None	None

Number of herds, 99.
Total number of animals tested, 3,073.
Incidence of positive reactors, 3.3 per cent.
*Comparable material also yielded positive results on nonglycerinated medium.

RESULTS

From the tissues examined from the 101 animals, tubercle bacilli were demonstrated in only 23, or approximately 22.8 per cent (table I). All of these positive results were obtained from tissues considered at necropsy to contain lesions of tuberculosis. In all instances the tubercle bacilli were found to be of the bovine type. Since there were 44 animals which at necropsy showed lesions considered to be those of tuberculosis, the diagnosis made at necropsy could not be confirmed by the laboratory in 21 instances. Tubercle bacilli were not demonstrated from the 17 so-called skin lesions or from the tissues from the 40 cases in which demonstrable lesions of tuberculosis were not found. Therefore, although 101 animals showed a sufficient sensitivity to tuberculin to be of diagnostic significance, tubercle bacilli were not demonstrated in the tissues of 78, or 77.2 per cent. The explanation for the tuberculin sensitivity in the latter group of animals probably constitutes the most important aspect of our present concept of the bovine tuberculosis problem.

Among the possible reasons for the failure to demonstrate tubercle bacilli in the 78 cases may be considered the following: (1) Our methods are necessarily inadequate. Cattle are large animals and the impossibility of examining every tissue minutely is obvious. (2) Sensitivity to tuberculin may have been due to dead tubercle bacilli, although this seems unlikely, or the sensitivity may have resulted from tubercle bacilli that eventually had become avirulent or nonviable. (3) Bacteria other than true tubercle bacilli may have been responsible.

Tuberculin Sensitivity. — As mentioned previously, all of the 101 animals from which material was obtained for laboratory study were tested with both avian and mammalian tuberculin. On the basis of the results of the laboratory and postmortem findings it will be convenient to separate the 101 cases into five groups for consideration of the tuberculin reactions.

Group 1 consisted of 23 animals from which tubercle bacilli were demonstrated. In this group the reactions to mammalian tuberculin were much more pronounced than the reactions to avian tuberculin. It is also important to mention that in nine instances no recognizable response to avian tuberculin occurred. Since bovine tubercle bacilli were obtained from tissues from each of the animals in this group, it should follow that the reactions to the homologous tuberculin should be more intense than the reactions to the heterologous product. The data substantiate this presumption. Since avian tuberculosis was present on but 2 of the 21 premises, the positive reactions to avian tuberculin were probably due to a reciprocal sensitivity rather than to a specific or homologous sensitivity.

Group 2 consisted of 17 animals with so-called skin lesions. Although neither tubercle bacilli nor other acid-fast bacteria were isolated from the lesions in the subcutaneous tissues in this group, the reactions to mammalian tuberculin were for the

most part pronounced and were sufficiently severe to be considered as definitely positive. Ten of the animals reacted positively to both avian and mammalian tuberculin; six reacted positively to mammalian tuberculin only and one animal reacted positively to avian tuberculin, but the reaction to mammalian tuberculin was negative. As in group 1, the most pronounced reactions occurred at the site of the injection of mammalian tuberculin. As a whole, the reactions to avian tuberculin were inferior to those elicited by the mammalian product. For the one instance in which there occurred a positive response to avian tuberculin and a negative reaction to mammalian tuberculin there is no apparent explanation. Tuberculous poultry were not present on the premises from which this animal came and, as implied previously, avian tubercle bacilli were not demonstrated from the tissues examined.

Group 3 consisted of 40 animals without demonstrable lesions of tuberculosis at the time of necropsy. Two features are of particular interest in the data concerning the animals comprising group 3. Although all of the animals reacted to mammalian tuberculin, 21 of the 40 animals failed to react to avian tuberculin and, among those that did react to it, the response with one exception was for the most part inferior to the reaction to mammalian tuberculin. It is also of interest to note that, although avian tuberculosis was present on but three of the premises from which the cattle in groups 1 and 2 came, this infection was present among the poultry on 11 of the 40 premises represented by the cattle in group 3. However, the significance of these differences is not apparent, since avian tubercle bacilli were not isolated from any of the animals.

Group 4 consisted of 21 animals with lesions that were considered tuberculous at the time of necropsy but from which viable tubercle bacilli could not be demonstrated. Eleven of the 21 animals had revealed a sensitivity to both avian and mammalian tuberculin, while eight failed to react to the avian product and two failed to respond to mammalian tuberculin. Microscopically no morbid changes were observed in the material examined. This fact, however, does not necessarily indicate that lesions were not present in those portions of the respective tissues that were prepared for the making of cultures and the inoculation of guinea pigs.

Group 5 consisted of 17 animals, 16 of which were included in the groups previously mentioned. This group is of special interest because of the fact that all of the lesions recognized microscopically were those associated with ray fungi.*

Microscopically lesions of tuberculosis were not observed, although at the postmortem examination the lesions in twelve of the animals were presumably considered as tuberculous. By laboratory procedures the diagnosis of tuberculosis was confirmed in only one case. The reactions of the animals in this group to avian and to mammalian tuberculin are summarized in table II. With one exception all of the animals had reacted to mammalian tuberculin while all but six had reacted to avian tuberculin. As may be noted, most of the reactions to avian tuberculins were of minimal severity and the most severe reactions to avian tuberculin occurred in the animal that failed to react to mammalian tuberculin. In this instance avian tuberculosis was known to have been present on the premises from which the animal came. Tuberculosis of the poultry was also established as being present on the premises from which the animals designated as cases 1, 4, 10 and 11 originated.

It may be of some importance to mention that the only instances in which more than one animal in a herd reacted to tuberculin occurred in group 1. In this group there were two herds, in each of which two positive reactors were observed. Among the remaining 97 herds, representing 2,820 cattle, only one reactor occurred in each herd.

DISCUSSION

Although this report leaves many important questions unanswered, it has disclosed a few facts and may be the impetus, we hope, for a more concerted attack on a

*Whether these lesions represented actinomycosis or actinobacillosis was not determined.

fundamental problem, namely, the possible existence of the factor or factors other than tubercle bacilli that may sensitize cattle to tuberculin. The problem is of great importance and success in its solution will be most likely when several laboratories and many competent workers are engaged in its investigation. To consider research in this phase of the tuberculosis problem as futile and likely to be unproductive is to admit our inability to grasp the significance of the problem or to assume that the obstacles are too many and too great to be surmounted. However, more work is imperative if we are eventually to find the explanation for a situation which provides a reasonable doubt as to the specificity of tuberculin as a means of detecting infections of cattle with tubercle bacilli. It is not impossible that other factors may be implicated, and progress in the understanding of the situation is most likely to occur if we assume an open-minded attitude rather than insist that a positive reaction to tuberculin is infallible as indicative of previous contact with one or more of the recognized types of the tubercle bacillus.

It does not seem likely that the tuberculin is at fault. The product prepared by the U. S. Bureau of Animal Industry is definitely dependable and of a high standard of purity. If a lack of specificity exists, this deficiency is probably inherent in all tubercle bacilli whose metabolism is requisite for the tuberculoprotein content of all tuberculins.

The significance of investigations of this character is perhaps difficult to appraise because of the limitations in the amount of material that can be utilized by a single laboratory. Whether or not comparable results might be obtained from material from other parts of the United States is problematic. However, under the restrictions imposed by the methods used, certain facts became apparent, the more important of which are not new. It has been recognized for a considerable time that a large percentage of cattle that react to tuberculin in areas where tuberculin testing has been conducted over a period of years will be without demonstrable morbid signs of tuberculosis when examined at necropsy. Furthermore, the fact that cattle infected with bovine tubercle bacilli may react to avian tuberculin has been estab-

TABLE II—Summary of 17 Cases Showing Granulomatous Lesions Associated with Ray Fungi (Group 5)

Cases	Lesions Found at Necropsy	Tuberculin Reaction				Tubercle Bacilli Demonstrated
		Avian		Mammalian		
		High*	Low†	High*	Low†	
1	Tuberculosis	Negative	Negative	x2	P_1	None
2	Tuberculosis	P_1	P_2	x3	P_2	None
3	Tuberculosis	P_1	P_1	P_2	P_2	None
4	Tuberculosis	P_1	Negative	P_3	P_3	None
5	Tuberculosis	x3	P_5	Negative	Negative	None
6	Tuberculosis	P_1	P_2	P_3	P_5	None
7	Tuberculosis	Negative	Negative	x3	P_5	Positive‡
8	Tuberculosis	Negative	Negative	x2	P_3	None
9	Tuberculosis	P_2	P_2	P_1	P_2	None
10	Tuberculosis	P_1	Negative	P_2	P_2	None
11	Tuberculosis	P_1	Negative	P_2	P_2	None
12	Tuberculosis	Negative	Negative	x3	P_2	None
13	"Skin lesion"	P_1	P_1	P_2	P_1	None
14	None	P_1	P_2	P_2	P_3	None
15	None	P_1	Negative	P_2	P_2	None
16	None	Negative	Negative	P_2	P_3	None
17	None	Negative	Negative	x3	P_2	None

*Tuberculin injected into caudal fold.
†Tuberculin injected into lateral aspects of vulva.
‡Tubercle bacilli of bovine type.

lished adequately by previous observations.[3, 4]

Our failure to obtain other than bovine tubercle bacilli from the material examined was somewhat surprising when it became known that tuberculosis was present among the chickens of 23 of the 99 premises from which the cattle originated. Although neither rabbits nor chickens were used in attempts to demonstrate the tuberculous character of any of the original material, if avian tubercle bacilli had been present it is reasonable to presume that these bacteria would have become evident by the culture method used. Regardless of the fact that avian tubercle bacilli were not demonstrated from the tissues examined, it would be presumptuous to claim that our findings eliminate avian tubercle bacilli as a possible factor in the tuberculin sensitization of cattle on premises where tuberculosis of poultry existed. However, this hypothesis is weakened definitely when one attempts to account for sensitivity to avian tuberculin on those premises where tuberculosis among the chickens did not occur. In these instances it suffices to say that the sensitization to avian and to mammalian tuberculin was probably the result of a factor or factors common to the two. Such a factor could be bovine or human tubercle bacilli or some undetermined bacterium that is perhaps widespread in its geographic distribution.

The question of what the sensitizing agent is remains unsettled and the justification for our present report must be sought in the value that may occur from emphasizing the importance of the fundamental problem, which simply stated is: Can cattle be sensitized naturally to a diagnostic dose of tuberculin by bacteria other than tubercle bacilli?

It is likely that cattle may be, and probably are in some instances, rendered sensitive to tuberculin by factors other than tubercle bacilli, but before one accepts this as true, there should be presented facts sufficient to make the claim convincing. Likewise, until proof to the contrary is available, it would seem prudent to assume an attitude of caution as regards the absolute specificity of tuberculin in so far as positive reactions in cattle are concerned. Many phases of this phenomenon remain to be investigated and, until we have more information, our position will be less assailable if we avoid dogmatic conclusions.

The study provided a means of comparing the relative value of cultural methods and animal-inoculation procedures in demonstrating bovine tubercle bacilli in clinical material. The nonglycerophilic character of most strains of the bovine tubercle bacillus when present in naturally acquired lesions in cattle was further confirmed. Of the 19 strains that produced recognizable growth on the nonglycerinated medium, only two grew in the medium that contained glycerin. The greater number of instances in which positive results were obtained by the inoculation of guinea pigs confirms the fact that animal inoculation is the method of choice in demonstrating mammalian tubercle bacilli from material in which the infective agents are few or obscure.

SUMMARY AND CONCLUSIONS

Attempts were made to demonstrate tubercle bacilli by culture methods and by the inoculation of guinea pigs with the tissues of cattle that had reacted to avian or to mammalian tuberculin, or to both of these products. The total number of cattle tested was 3,073, comprising 99 separate herds in the state of South Dakota. One hundred and one animals reacted positively to the test (3.3 per cent) and were slaughtered. Lesions considered at necropsy to be those of tuberculosis were present in 45 carcasses, so-called skin lesions only were found in 16, while in 40, or approximately 39.6 per cent, no lesions of tuberculosis were observed. In 23 instances, all representing cattle in which lesions of tuberculosis were found at necropsy, tubercle bacilli were demonstrated. All strains were proved by animal pathogenicity tests to be of the bovine type.

[3]Feldman, W. H.: Avian Tuberculosis Infections (The Williams and Wilkins Company, Baltimore, 1938), p. 302.

[4]Feldman, W. H., and Karlson, A. G.: The pathogenicity for cattle of the avian tubercle bacillus. Proc. 43rd Ann. Meet. U. S. Live Stock San. Assn. (Chicago, 1939), pp. 114-132.

Although tuberculosis was present in the poultry on 23 of the 99 premises, the avian tubercle bacillus was not obtained from any of the tissues examined. From the data obtained it is concluded:

1) The previously recognized fact that many cattle presumably sensitized by bovine tubercle bacilli will react to avian tuberculin administered intracutaneously has been confirmed. In most instances, however, the reactions are less pronounced to avian tuberculin than to mammalian tuberculin.

2) Among animals that react positively to either avian or mammalian tuberculin there occur a considerable number in which by the methods available the agent responsible for the sensitization can not be determined or is obscure.

3) The possibility that agents other than tubercle bacilli are capable of sensitizing cattle to tuberculin should be reëxamined.

4) The agent responsible for detectable lesions of tuberculosis in cattle in the great majority of instances is the bovine tubercle bacillus. The avian and the human types of the organism are of minimal importance.

5) Apparently it is difficult at the time of the necropsy to differentiate in some instances lesions of tuberculous adenitis from minimal lesions of adenitis due to ray fungi infections.

6) Regardless of possible shortcomings, the intradermic tuberculin test when properly applied and intelligently interpreted still remains the most reliable single method for the detection of infections of cattle due to the tubercle bacillus.

The Nerve Supply to the Bovine Mammary Gland*

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MUCH HAS BEEN written about the endocrine influence upon the growth and development of the mammary gland and upon milk secretion. A considerable amount of research has been done on the nervous influence upon the secretion and excretion of milk. The histologic structure of the udder and teat at various stages of functioning has been determined in many animals. In general, the gross structure and the gross blood supply are well known. Very little exact and conclusive information can be found in the literature, however, concerning the nerve supply to the mammary gland of the cow.

In this investigation an attempt has been made to establish in detail the innervation of the bovine mammary gland. The approach is mostly anatomic, but it is supplemented by physiologic experimentation.

MATERIAL AND METHODS

Eighteen female and four male cadavers were dissected to trace the nerves to the udder. The lumbosacral plexus and the sympathetic nerves in the posterior part of the body were investigated. Histologic sections were made from several udders to search for nerve endings and ganglia. The course of the nerves to the mammary vessels was determined. Before the cows were killed and embalmed, the nerves to the udder were divided at various places to locate the sensory area of distribution of each nerve. The peripheral end of the cut inguinal nerve was stimulated with a current in an attempt to cause the secretion of milk and vasoconstriction. Pilocarpine also was used to try to produce milk secretion.

NERVES TO THE UDDER

There are four sources of the nerve supply to the bovine mammary gland: inguinal, ventral branches of the first two lumbar, perineal, and sympathetic nerves.

1) The inguinal nerve is formed by the ventral branches of the second, third and fourth lumbar nerves (fig. II). The third ramus is always a component but each of the others may not always contribute to its origin. They emerge between the two psoas muscles in a plexiform manner. Finally two nerves are formed which course downward and backward, one above and one below the cirumflex iliac artery (fig. I, 18 and 19). They descend outside the peritoneum toward the internal inguinal ring anterior to the external iliac vessels. They usually unite just before entering the internal inguinal ring to form the inguinal nerve. They may remain separated, however, and merely send branches to each other. The inguinal nerve divides at the internal inguinal ring into anterior and posterior inguinal nerves (fig. I, 22 and 23). The anterior inguinal nerve emerges from the very anterior part of the external inguinal ring or through the aponeurosis of the musculus obliquus abdominis externus near it, and is distributed to the fold of the flank and the anterior part of the fore quarter and the anterior part of the fore teat with its apex. The anterior inguinal nerve may be double. It supplies the glandular tissue only slightly. The posterior inguinal nerve traverses the inguinal canal in front of the external pudic vein. Just outside the external inguinal ring it gives off one or two small anterior branches which supply the glandular tissue somewhat, but mainly the skin of the lateral part of the anterior quarter as far posteriorly as the interquarter junction (fig. I, 24). These branches also supply the posterior portion of the front teat. The posterior or main portion of the posterior inguinal nerve is the only part of the inguinal nerve that makes an S-curve with the external pudic vessels (fig. I, 25). It gives a small branch to the supramammary

*From the Department of Veterinary Anatomy, Veterinary Division, Iowa State College. For the entire work and references see "The Nerve Supply to the Bovine Mammary Gland," Master's Thesis, Library, Iowa State College (1940).

lymph-node area and is distributed with the vessels to the glandular tissue of the posterior quarter and the posterior portion of the anterior quarter. It also supplies the rear teat and the skin of the rear quarter, except posteriorly above the base of the teat. The fibers of distribution of this nerve usually traverse the glandular tissue before becoming cutaneous.

2) The ventral branches of the first and second lumbar nerves pass above the psoas muscles and descend in the flank between the m. transversus abdominis and m. obliquus abdominis internus to perforate the rectus and the rest of the abdominal wall just laterally to the median line and above the anterior quarter of the udder (fig. I, 15 and 16). After perforating the wall they run forward before innervating the very anterior part of the udder. The second lumbar nerve frequently perforates the glandular substance of the fore quarter. They supply the skin mainly. As the first and second lumbar nerves descend in the flank, they give off superficial branches at the level of the hip joint. These branches, however, do not go to the udder (fig. I, 15b and 16b).

3) The perineal nerve is a small branch from the pudic nerve. It comes over the ischial arch to descend in the perineum to supply the skin of the posterior part of the udder as far down as the base of the posterior teat (fig. I, 28; fig. II, 9).

4) The sympathetic trunk runs along the sides of the bodies of the vertebrae in the thoracic area, but becomes ventral in the sacral region. The trunk becomes smaller as the posterior portion of the body is reached and terminates at the emergence of the last coccygeal nerve. The trunks of the opposite sides anastomose in the sacral region and often unite in the coccygeal area and then divide again before terminating. They usually appear as a number of fasciculi instead of one bundle. For each spinal nerve there is a ganglion on the sympathetic trunk (fig III, 14); more posteriorly, however, this varies.

Connecting the ventral ramus of each spinal nerve to the sympathetic trunk at or near the ganglia are rami communicantes (fig. III, 9). Several of these (up to five or six) are present at each spinal nerve. They join the spinal nerve just lateral to the trunk made by the union of the two spinal nerve roots. The rami communicantes are of two types, gray and white. The white ones consist mostly of myelinated fibers, while the gray ones are mostly nonmyelinated. Both white and gray fibers are usually represented in the same ramus.

The cell bodies of the sympathetic nerves are located in the lateral horn of the gray matter of the spinal cord in the thoracicolumbar area. They leave the cord by way of the ventral roots of the spinal nerves, course to the ventral spinal rami and then leave the spinal nerves as white rami communicantes to join the sympathetic trunk at the respective ganglia (vertebral or lateral ganglia). Around the origin of the celiac, anterior mesenteric and posterior mesenteric arteries are ganglia also (prevertebral or collateral). They receive fibers from the thoracic sympathetic trunk as the splanchnic nerves and directly from the sympathetic trunk.

The pelvic organs receive fibers directly from the sympathetic trunk. Between the origin in the cord and the visceral structure innervated are two fibers with a synapse between them occurring either in the vertebral or prevertebral ganglion, but not in both. The fiber from the cord to the synapse in the ganglion is called preganglionic and the one from the synapse to the structure innervated is called postganglionic. Postganglionic fibers originating in the vertebral ganglia go by way of the gray rami communicantes to the spinal nerves and thus to the smooth muscle, glands and vessels of the skin, or to abdominal viscera. Postganglionic fibers originating in the prevertebral ganglia course to the abdominal viscera, but not to the spinal nerves. Parasympathetic fibers from the dorsal esophageal trunk of the vagus join the sympathetic fibers around the celiac and anterior mesenteric arteries to form plexuses. Subsidiaries of these plexuses are distributed to each organ in the abdominal cavity.

The sympathetics in general throughout

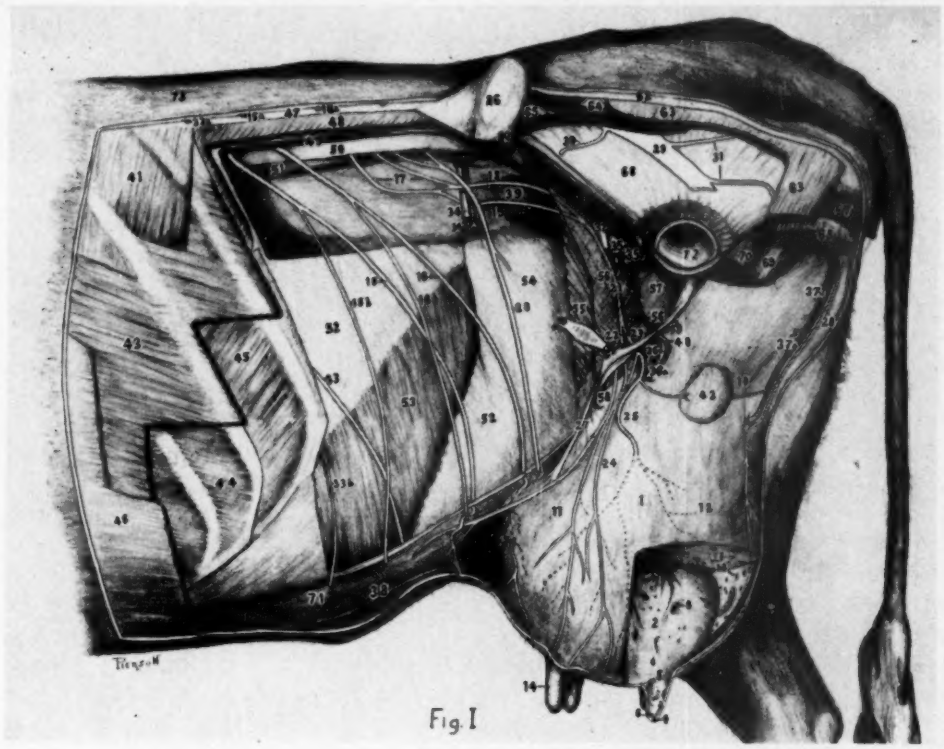

Fig. I

1. Udder
2. Lactiferous sinus
3. Dilated portion of teat cistern
4. Streak canal
5. Shelf at base of teat
6. Folds of rosette
7. Large milk-collecting duct
8. Trabecula
9. Fascia at anterior limit of udder
10. Lateral suspensory ligament of udder
11. Anterior quarter of udder
12. Posterior quarter of udder
13. Glandular substance (cut)
14. Teat
15. Ventral branch of first lumbar nerve
15a. Dorsal branch of first lumbar nerve
15b. Superficial branch of first lumbar nerve
16. Ventral branch of second lumbar nerve
16a. Dorsal branch of second lumbar nerve
16b. Superficial branch of second lumbar nerve
17. Branches of origin of inguinal nerve
18. Dorsal component of inguinal nerve
19. Ventral component of inguinal nerve
20. Lateral cutaneous nerve
21. Inguinal nerve deep to inguinal ligament
22. Anterior inguinal nerve
23. Posterior inguinal nerve
24. Anterior branch of posterior inguinal nerve
25. Main branch of posterior inguinal nerve
26. Tuber coxae
27. Cut edge of inguinal ligament
28. Perineal nerve
29. Sciatic nerve
30. Anterior gluteal nerve
31. Posterior gluteal nerve
32. Femoral nerve
33. Ventral branch of last thoracic nerve
33a. Dorsal branch of last thoracic nerve
33b. Superficial branch of last thoracic nerve
34A. Circumflex iliac artery
34V. Circumflex iliac vein
35A. Femoral artery
35V. Femoral vein
36A. External pudic artery
36V. External pudic vein
37A. Perineal artery
37V. Perineal vein
38. Subcutaneous abdominal or "milk" vein
39. Deep inguinal lymph node
40. Supramammary lymph duct entering inguinal canal
41. M. serratus dorsalis posterior
42. Supramammary lymph node
43. M. obliquus abdominis externus
44. Mm. intercostales externi
45. Mm. intercostales interni
46. M. cutaneous trunci
47. Lumbodorsal fascia—cut border
48. M. obliquus abdominis internus
49. M. quadratus lumborum
50. M. psoas major
51. M. psoas minor
52. Aponeurosis of m. transversus abdominis
53. Muscular part of m. transversus abdominis
54. Peritoneum covered by transverse fascia
55. M. obliquus abdominis internus—cut border
56. Posterior border of m. obliquus abdominis internus
57. Aponeurosis of m. obliquus abdominis externus—inguinal ligament
58. Anterior lateral angle of external inguinal ring

59. Posterior medial angle of external inguinal ring
60. M. tensor fasciae latae
61. M. ilio psoas
62. Gluteal fascia
63. M. biceps femoris
64. M. gluteus medius
65. M. gluteus accessorius
66. M. gluteus profundus
67. M. semitendinosus
68. M. semimembranosus
67. M. gracilis
70. M. adductor
71. Aponeurosis of m. obliquus abdominis externus
72. Acetabulum
73. Skin

the body are distributed by way of the blood vessels. Afferent fibers arise from the viscera and travel centrally with the sympathetics by way of the white rami communicantes to the cord. They also follow the sacral parasympathetics and the phrenic nerve. They do not form synapses in the sympathetic ganglia, however. The sympathetic system is efferent. White rami communicantes were found by histologic examination to be present in conclusive numbers as far posteriorly as the third lumbar segment. Parasympathetics have not been proved to be present in cutaneous areas. They arise from the central nervous system in the cranial and sacral areas and course with the third, seventh, ninth and tenth cranial nerves and with the visceral branches of the second, third and fourth sacral nerves to the viscera. This system is also efferent. The synapses and ganglia (terminal ganglia) between the preganglionic and postganglionic fibers of the parasympathetic system are located in or near the structure innervated. Like the sympathetics, only one synapse is present between the fiber origin and the distribution to the visceral structure.

The cutting of the inguinal nerve caused vasodilatation to occur in the udder with an increase in local temperature. The removal of the sympathetic trunk in the lumbar area produced the same effect. However, the removal of the posterior mesenteric plexus and ganglion did not cause vasodilatation. The sympathetics are, therefore, vasoconstrictors to the peripheral blood vessels and do not course by way of the posterior mesenteric plexus to the udder, as was commonly believed. The sympathetic fibers travel with the spinal nerves to the periphery before coming in contact with the mammary vessels. Few if any fibers follow the external iliac vessels from the sympathetic trunk and hypogastric plexuses continuously to the udder. Severing of the inguinal nerve near its origin caused the nerves to the mammary vessels to degenerate.

Since the mammary gland develops as a cutaneous gland, it should be innervated like cutaneous structures. Because parasympathetics can not be proved to be present in the skin, none should be found in the mammary gland. Since ganglia were not found in the bovine udder, the author concluded that the mammary gland does not have a parasympathetic supply.

PHYSIOLOGY

The cow apparently lets down her milk by the contraction of the smooth muscle in the udder. The muscle is arranged around the lobules in such a manner as to cause a pushing out of the milk contained in the alveoli and ducts. One cubic centimeter of obstetrical pituitrin given intravenously will cause milk to run out of the teats (in which teat tubes have been inserted) in streams. Adrenalin fails to produce such an effect. The fact that pituitrin acts directly upon smooth muscle while adrenalin acts through the sympathetic nerves may explain their difference in action on the mammary muscle fibers. The posterior pituitary gland by its secretion of pituitrin then causes the excretion of milk. The influence of the sympathetic nerves on the smooth muscle of the udder can not be denied, however, even though the hormonal control overshadows the nervous control. Smooth muscle seems to have the power to continue to function although it has been denervated. It is believed there may be an intrinsic neuromuscular factor present. Tone returns to the smooth muscle of the denervated arteries after a few days.

This has been explained as the action of

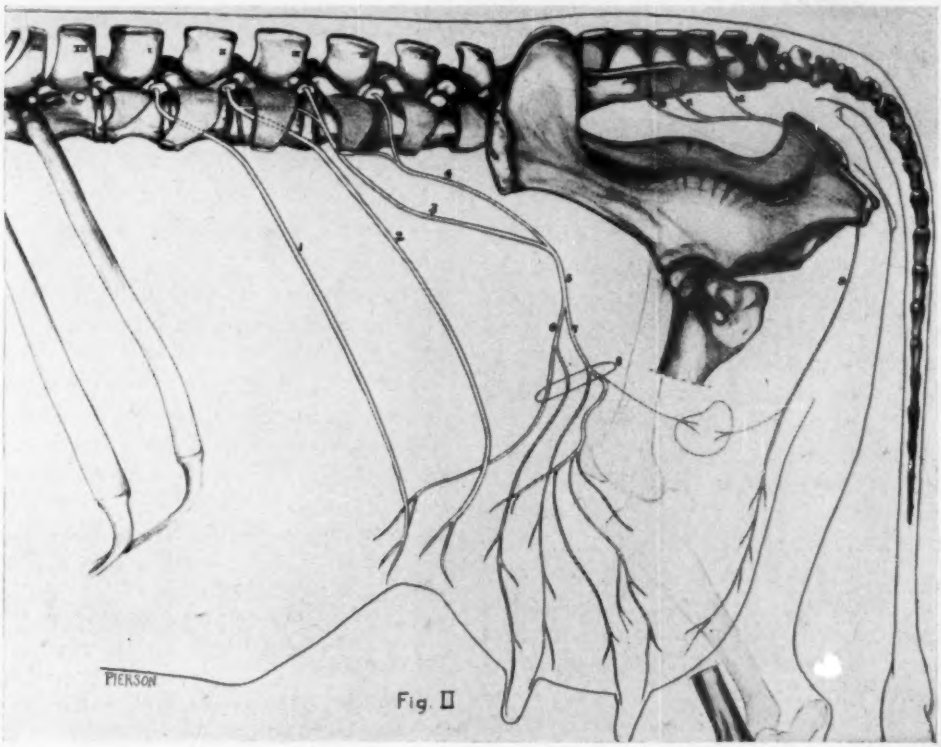

Fig. II

1. Ventral branch of first lumbar nerve
2. Ventral branch of second lumbar nerve
3. Ventral component of inguinal nerve
4. Dorsal component of inguinal nerve
5. Inguinal nerve
6. Anterior inguinal nerve
7. Posterior inguinal nerve
8. External inguinal ring
9. Perineal nerve
10. Second sacral origin of pudic nerve
11. Third sacral origin of pudic nerve
12. Fourth sacral origin of pudic nerve

some chemical substance which can influence the tone of blood vessels. An udder which has been stripped of sympathetic influence can still function. The afferent nerves to the udder and teats are necessary, however, since those impulses cause the anterior pituitary gland to secrete the lactogenic hormone, the absence of which causes the cessation of milk secretion.

NERVES IN THE UDDER

The nerves to the gland substance run in the connective tissue. None was found by histologic sections to end in or on the alveolar cells. No increase in milk secretion was noted when pilocarpine or inguinal nerve stimulation was employed. The udder was first rendered as free as possible of milk by the insertion of teat tubes and intravenous injection of 1 cc. of obstetrical pituitrin. The nerves to the udder were not plentiful and probably are not secretory. It would seem unreasonable that a gland which changes so much, depending on its state of function, would be dependent on a nerve supply for secretion. The control is hormonal.

LUMBOSACRAL PLEXUS AND PUDIC NERVE

The lumbosacral plexus is formed by the ventral branches of the last three lumbar and first two sacral spinal nerves. The femoral and obturator nerves usually arise from the ventral rami of the fourth, fifth and sixth lumbar nerves (fig. III, 17 and 18). The sciatic nerve arises from the sixth lumbar and the first and second sacral ventral branches (fig. III, 20). The anterior gluteal nerve originates from the sixth lumbar and first sacral rami (fig. III, 19). The posterior gluteal nerve arises from the first and second sacral and often

the sixth lumbar nerves (fig. III, 7). The pudic nerve arises from the third and fourth and often the second sacral ventral spinal branches (fig. II, 10, 11, 12; fig. III, 8).

DORSAL SPINAL NERVES

The dorsal rami of the spinal nerves are smaller than the ventral. The sixth lumbar dorsal ramus is very small. The first three lumbar dorsal branches innervate the flank as far ventrally as the fold, but these dorsal branches do not innervate the udder.

The nerves to the mammary gland are the same in both sexes except as to the distribution to the udder or scrotal area.

ANESTHESIA OF THE UDDER

Since it is desirable to anesthetize a part by applying the anesthetic at some distant point, the udder can be blocked very efficiently by employing the paravertebral lumbar method of Farquharson.[1] For teat operations the second, third and fourth lumbar nerves must be blocked paravertebrally on the side of the involved teat. To anesthetize the whole gland for amputation, the first lumbar nerve also should be blocked to get the skin at the very anterior part of the udder. The perineal nerve also is involved

[1]Farquharson, J.: Paravertebral lumbar anesthesia in the bovine species. J.A.V.M.A., xcvii (1940), pp. 54-57.

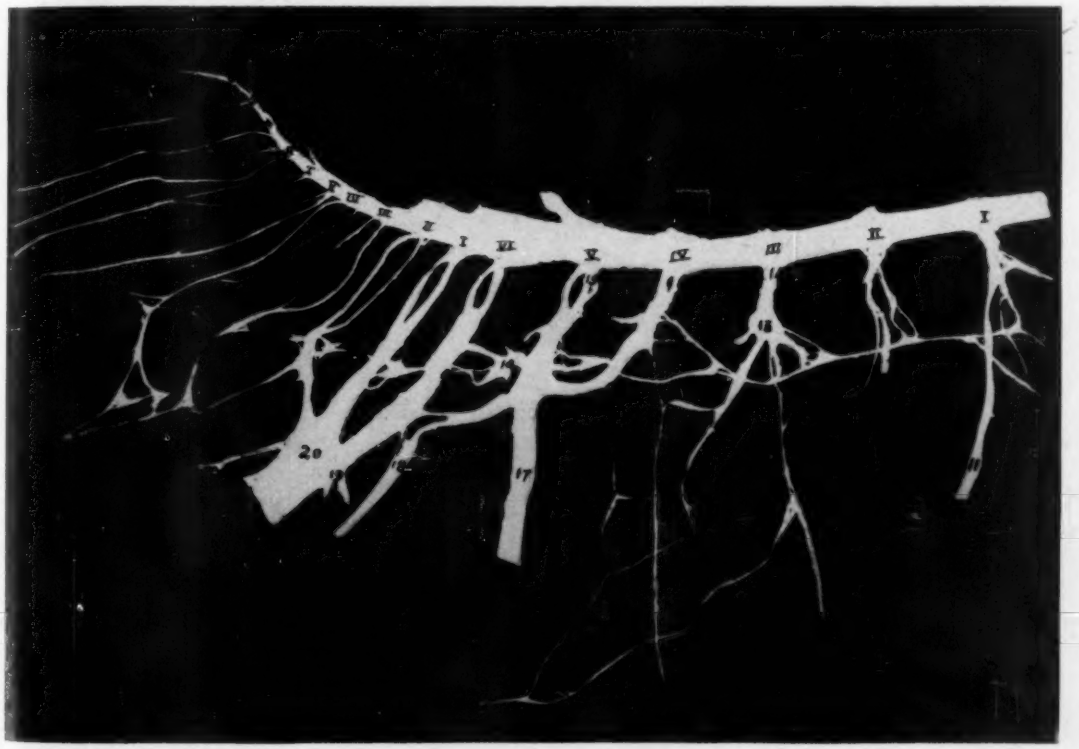

FIG. III

1. Inguinal nerve
2. Dorsal component of inguinal nerve
3. Ventral component of inguinal nerve
4. Lateral cutaneous nerve
5. Cutaneous branch from ventral inguinal component
6. Sympathetic trunk
7. Posterior gluteal nerve
8. Pudic nerve
9. Rami communicantes
10. Dorsal branch of first lumbar nerve
11. Ventral branch of first lumbar nerve
12. Ventral branch of second lumbar nerve
13. Dorsal root ganglion
14. Sympathetic ganglion
15. Dorsal root of spinal nerve
16. Ventral root of spinal nerve
17. Femoral nerve
18. Obturator nerve
19. Anterior gluteal nerve
20. Sciatic nerve

in this case as it supplies the posterior portion above the rear teats. It can be reached by making the injection just below the vulva close to the median line. More fluid can be used here to insure reaching the nerve. Of course, both sides must be blocked for amputation.

The portions of the second, third and fourth lumbar nerves which make up the inguinal nerve course ventrally between the two psoas muscles. To block them the anesthetic must be injected close to the respective intervertebral foramina. Figure II shows where the nerves leave the vertebral canal.

SUMMARY

1) The udder is innervated by sensory and sympathetic fibers.

2) The sensory and sympathetic fibers reach the udder by way of the inguinal, first two lumbar ventral branches and the perineal nerves.

3) The inguinal nerve arises from the second, third and fourth lumbar ventral spinal branches.

4) No secretory nerves were found in the udder.

5) Gray rami communicantes are present in conclusive numbers as far posteriorly as the third lumbar segment.

6) The sympathetics are vasoconstrictor to the vessels and motor to the smooth muscle of the udder. They reach the spinal nerves by means of the gray rami communicantes and not by way of the posterior mesenteric plexus.

7) The nerves to the mammary blood vessels travel by way of the spinal nerves and not along the main vessel trunks to reach the gland.

8) The lumbosacral plexus is formed by the ventral branches of the last three lumbar and first two sacral nerves.

9) The pudic nerve, which continues to the udder as the perineal nerve, arises from the second, third and fourth sacral ventral rami.

10) The udder can be anesthetized readily by the paravertebral lumbar method.

Intestinal Pathology in Experimental Bovine Esophagostomiasis*

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IN A PREVIOUS paper[1] the writers have called attention to the fact that nodular lesions associated with the larval stages of *Oesophagostomum radiatum* in the intestinal wall of cattle were first discovered by Drechsler[2] in 1876. The nematodes recovered at that time, however, were considered to be the larvae of an unknown strongyle. Eighteen years later Ströse[3] studied the disease produced by these parasites, but attributed the condition to the larvae of a hookworm which he called *Ankylostomum bovis*. In 1908, Marotel[4] gave the first detailed description of similar verminous lesions observed in the intestine of naturally infected European cattle and showed for the first time that these lesions were actually due to larvae of *O. radiatum*.

Since 1908, the gross pathology associated with nodular worm infection in cattle has been observed often in naturally acquired cases of esophagostomiasis. Unfortunately, however, because of the circumstances under which these cases occurred, other species of parasites and bacteria could not be entirely eliminated as factors affecting the pathologic picture associated with nodular worm infection. Since, as far as is known to the writers, there is no record in the literature on the pathologic changes produced by larvae of *O. radiatum* following their artificial administration to healthy calves, the following data obtained during a recent study of experimental bovine esophagostomiasis are reported.

Materials and Methods

Nine calves (eight infected and one control) were used in this investigation. They were born on dairy farms in the vicinity of San Juan, and were from 2 to 3 months old when first infected. They were raised in concrete pens with wooden floors which were cleaned twice daily. They were fed cow's milk and powdered skim milk in water from a bucket, commercial calf meal, and cured alfalfa and timothy hay, so as to prevent infection with extraneous parasites.

The larvae of *O. radiatum* which were administered to the experimental calves were obtained by culture from nodular worm eggs passed in the feces of a previously parasite-free calf which had been artificially infected with *O. radiatum*. The feces containing the nematode eggs were mixed with animal charcoal and the mixture allowed to stand for seven days at room temperature in large culture dishes. The infective nodular worm larvae recovered from the cultures by means of the Baermann apparatus were counted and administered by the mouth to the experimental calves by means of a funnel and rubber tube according to the dose rate and on the dates indicated in table I.

The experimental calves were found to be free from parasites at the beginning of the experiment, as indicated by fecal examination. They were killed after artificial infection on the days indicated in the table. The number and species of parasites recovered *post mortem* and the character of the pathologic changes in the intestines were noted. Sections of the intestinal wall showing pathologic changes were fixed in Zenker's fluid and submitted to the Department of Pathology of the School of Tropical Medicine for histologic examination.

Data on the Infection of Experimental Calves

The data on the number of larvae administered to the experimental calves, the date of the postmortem examination, the number of days between the administration of larvae and autopsy, and the number and

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[1]Andrews, J. S., and Maldonado, J. F.: The life history of *Oesophagostomum radiatum*, the common nodular worm of cattle. Res. Bul. 2, Puerto Rico Agr. Exp. Sta. (1941). (In press.)

[2]Drechsler, G.: Ueber einen neuen Parasiten in der Schleimhaut des Rinderdarmes. Deut. Zeit. f. Thiermed. u. Vergl. Path., ii (1876), pp. 355-358.

[3]Ströse: Ueber eine Ankylostomumlarve (Ankylostomum S. (*Dochmius bovis*, N.Sp.) im Dünndarme des Rindes. Deut. Zeit. f. Theirmed., xxi (1894), pp. 110-114.

[4]Marotel, G.: L'oesophagostomose nodulaire. J. Med. Vet. (1908), pp. 522-534, 591-604.

TABLE I—Data on the Infection of Experimental Calves and the Number and Species of Parasites Recovered Post Mortem

Calf No.	Days on Which Larvae Were Administered	Size of Dose	Total Number of Larvae Administered	Date of Autopsy	Number of Days Between Last Dose of Larvae and Autopsy	Parasites Recovered				
						Oesophagostomum Radiatum		Haemonchus Contortus	Strongyloides Papillosus	Bunostomum Phlebotomum	Trichuris Ovis
						No.	Per Cent	Number	Number	Number	Number
Single Infections											
8	11/10/39	17,000	17,000	11/13/39	3	Not counted		0	0	0	0
15	2/ 9/40	20,000	20,000	2/14/40	5	Not counted		0	0	0	0
10	11/15/39	52,000	52,000	11/25/39	10	8,800	16.92	0	0	0	0
9	12/ 1/39	35,000	35,000	12/20/39	19	2,505	7.13	0	0	0	0
14	2/ 9/40	20,000	20,000	2/12/40	32	3,200	16.00	0	0	0	0
Multiple Infections											
3	8/10/39-9/9/39	5,000 (daily)	155,000	9/12/39	3	16,000	10.32	714	1	9	0
2	7/11/39	9,000									
	7/13/39	4,000									
	7/15/39	12,000									
	7/21/39	28,000									
	7/24/39	60,000									
	8/28/39	20,000									
	9/ 2/39	30,000	163,000	10/19/39	47	154	.09	0	1	4	1
5	6/ 6/39	5,000									
	6/10/39	3,300									
	6/13/39	3,000									
	6/14/39	2,600									
	6/22/39	2,500									
	6/24/39	6,400									
	6/27/39	13,600									
	6/29/39	17,600									
	7/ 1/39	29,500									
	7/ 7/39	14,000									
	7/ 8/39	25,000									
	8/ 4/39	128,000	260,500	2/ 2/40	182	43	.02	0	0	11	0
Control Calf											
4	.....	.....	.....	9/18/39		15	.....	0	0	1	12

species of parasites recovered are shown in table I.

Six of the calves listed in table I, namely, Nos. 10, 9, 14, 3, 2 and 5, exhibited symptoms of gastrointestinal disturbance either during or after the period of infection. Calves 8 and 15 were autopsied before the appearance of symptoms and calf 4, the control, did not show symptoms of infection. The data in table I show that the larvae of *O. radiatum* were able to establish themselves in the calves as indicated by the recovery of 7.13 to 16.92 per cent of the larvae administered from the intestines of those calves autopsied within 33 days after the first infection. The smallness in number of nodular worms recovered from calves 2 and 5 was probably due to the loss of parasites between the period of experimental infection and the postmortem examination, and to the very small percentage of larvae returning to the lumen of the intestine after their migration into the mucosa. The number of parasites recovered from these two calves, therefore, does not give a true picture of the pathologic condition of the intestine in these animals.

The data also show that there were a few extraneous parasites in calves 2, 3, 4 and 5, and a few specimens of *O. radiatum* in control calf 4. These parasites, with the exception of the *Haemonchus contortus* in calf 3 and the *O. radiatum* in calf 4, were undoubtedly picked up during the period the calves spent with their mothers prior to being put in a clean pen. The *H. contortus* infection in calf 3 was due to

a slight contamination of the doses of *O. radiatum* larvae administered to this calf. The *O. radiatum* infection in calf 4 resulted from the ingestion of a few infective larvae of this species which were present in the Baermann residues given to this calf as a control measure at the time the remaining calves were infected, in order to determine whether or not some other organism present in the feces cultures besides nodular worm larvae might be responsible for the symptoms observed in the infected animals. In no case did the extraneous parasites encountered interfere with the interpretation of the data herein presented.

Lesions Produced by O. Radiatum

The number and distribution of nodular lesions produced by the larvae of *O. radiatum* in the intestines of the experimentally infected calves (with the exception of calf

TABLE II—Number and Distribution of Nodular Lesions Produced by the Larvae of O. Radiatum in the Intestines of Experimentally Infected Calves

Calf No.	Calves Receiving 1 Dose of Larvae				Calves Receiving More Than 1 Dose of Larvae			Control Calf
No. Feet from Abomasum	8	9	10	14	2	3	5	4
1 - 3	2	42	0	3	0	8	0	0
3 - 6	4	21	0	2	0	20	0	0
6 - 9	2	17	0	0	0	1	3	0
9 - 12	2	3	0	0	0	3	1	0
12 - 15	2	0	0	0	0	9	0	0
15 - 18	3	3	0	0	0	3	0	0
18 - 21	7	0	0	0	0	1	0	0
21 - 24	2	0	0	0	0	0	3	0
24 - 27	2	0	0	0	0	1	0	0
27 - 30	1	0	0	0	0	6	0	0
30 - 33	4	0	0	0	0	1	0	0
33 - 36	3	0	0	0	1	21	0	0
36 - 39	48	0	0	0	1	25	2	0
39 - 42	152	1	17	0	2	420	1	0
42 - 45	221	0	100	0	2	1,382	1	0
45 - 48	200	0	250	0	10	1,382	5	0
48 - 51	200	1	353	0	50	1,382	1	0
51 - 54	200	167	1,980	0	50	1,342	15	0
54 - 57	216	300	3,800	0	120	1,681	23	0
57 - 60	240	200	.....	1	200	1,681	44	0
60 - 63	62	200	.....	4	212	1,681	61	0
63 - 66	.....	400	.....	2	237	1,681	50	0
66 - 69	.....	300	.....	3	267	1,681	32	0
69 - 72	.....	0	.....	1	307	1,681	27	0
72 - 75	.....	0	.....	4	300	560	23	0
75 - 78	.....	0	.....	6	288	.....	28	0
78 - 81	.....	.....	.....	10	266	.....	13	..
81 - 84	.....	.....	.....	15	203	.....	4	..
84 - 87	.....	.....	.....	12	75	.....	9	..
87 - 90	.....	.....	.....	15	65	.....	....	..
90 - 93	.....	.....	.....	25	40	.....	....	..
Nodules in small intestine	1,573	1,655	6,500	103	2,696	16,653	346	0
Nodules in cecum	100	2,600	13,500	0	400	3,200	92	0
Nodules in colon	0	1,000	0	0	102	0	210	0
Nodules in rectum	0	0	0	0	400	0	0	0
Total nodules	1,673	5,255	20,000	103	3,598	19,853	648	0
Nodules in terms of per cent of larvae administered	9.85	15.01	38.47	.52	2.21	12.81	.25	0

15, in which they could not be counted) are recorded in table II.

The data in table II show that the majority of the nodular lesions occurred in the latter half of the small intestine and in the cecum and colon. Calf 14 presented lesions only in the small intestine. In addition, calves 9, 2 and 5 showed lesions in both the cecum and colon, and calf 2 presented lesions as far back as the rectum. The number of nodules expressed in terms of the percentage of larvae administered varied from 9.85 to 38.47 per cent in those calves autopsied within 20 days of the completion of infection. It will be noted that the percentage of larvae represented by nodules in calves 14, 2 and 5—namely, 0.25, 2.21 and 0.52 per cent, respectively—was much smaller than in the other calves. The small percentage of nodules found in these three calves was probably due to the delay of 32, 47 and 182 days, respectively, between the administration of the doses of larvae and the postmortem examination. These findings will be discussed later in connection with observations on the development of acquired resistance to nematode infections.

GROSS PATHOLOGY AND HISTOPATHOLOGY OF SINGLE AND MULTIPLE EXPERIMENTAL INFECTIONS. I. OBSERVATIONS ON SINGLE INFECTIONS

General Histopathology. — The larvae were found in the ileum and cecum, extending from the mucosa through the muscularis into the submucosa, wholly in the submucosa, and in the outer muscular coat. In the last-mentioned location they were always found within blood or lymphatic channels, and were evoking no reaction on the part of the vessel wall or surrounding tissues. The principal inflammatory changes took place in the submucosa and for the most part were focal in character, centering about larvae that were being destroyed.

Seventy-Five Hours After Infection.—Gross pathology: Wherever the larvae had entered the intestinal wall there were small, raised areas about 1 mm. in diameter, some of which were dark. There was no extensive general inflammation or irritation in the surrounding tissues.

Histopathology: The early alterations were characterized by a feeble histiocytic proliferation and infiltration with lymphocytes and lesser numbers of plasma cells about the larvae. A small amount of hemorrhage was observed about some of the parasites.

Five Days After Infection.—Gross pathology: The surface of the mucosa was dotted with tiny red spots approximately 1 mm. in diameter in which larvae were found. There was no widespread inflammation or irritation in the intestine.

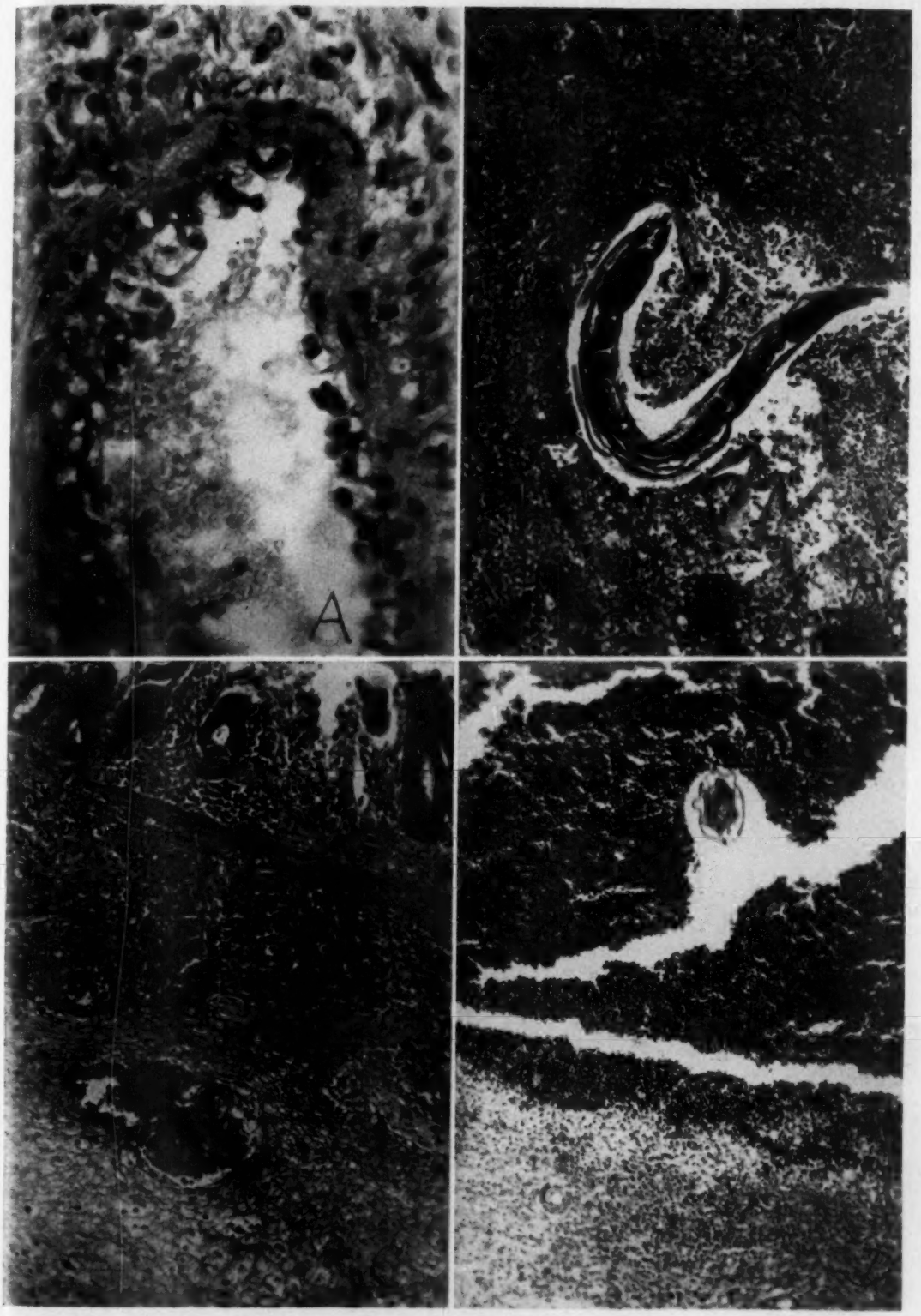
Histopathology. There was observed a slight, rather diffuse edema of the submucosa, and some of the small blood vessels (fig. 1A) near the inflammatory foci showed swelling, vacuolation and proliferation of the endothelial cells with deposition over the intima of a thin layer of fibrin, but without thrombosis. The histiocytic reaction and round-cell infiltration of the submucosa were more marked and extended along blood vessels as far out as the inner muscular coat.

Ten Days After Infection.—Gross pathology: The nodular lesions were now about 2 mm. in diameter. Some of them were hemorrhagic and many of them were pitted, indicating that the larvae within these particular nodules had escaped to the lumen of the intestine. Some of the nodules appeared to be full of pus and fatty material and there was extensive inflammation and edema of the intestinal wall and of the mesentery. The cecum was much inflamed and thickened.

Histopathology. The height of the pathologic process was reached at this time and was characterized by the formation of abscesses (fig. 1B) containing larvae and polymorphonuclear leucocytes, and surrounded by a poorly defined zone of epithelioid cells. Larvae were often found within veins in the submucosa. The small veins near abscesses were at times partially occluded by clumps of fibrin (fig. 1C). At this stage the edema and round-cell infiltration extended to the subserosa. A mesocolic lymph gland showed hyperplasia of lymphoid

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE—FIG. 1

A) Blood vessel, showing proliferative changes in endothelium five days after infection (x 450).
B) Abscess about larva ten days after infection (x 100).
C) Fibrin clumps in small veins; edema and round-cell infiltration of submucosa (x 100). Same calf as B.
D) Abscess composed of eosinophiles about dead larva three days after administration of thirty-first daily dose of 5,000 larvae. Narrow peripheral zone of histiocytes and fibroblasts (x 100).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tissues and presented three abscesses not over 2 mm. in diameter, and without a peripheral zone of epithelioid cells. A larva was visible within one of these abscesses. Special staining by the Gram method did not disclose bacteria in the abscesses. Only in one small area of necrosis in the submucosa of one animal was a mixed bacterial growth of Gram-positive and Gram-negative cocci and bacilli encountered.

Nineteen Days After Infection.—Gross pathology: The intestinal wall was still inflamed and edematous. The nodules had decreased somewhat in size, measuring at this time about 1.5 mm. in diameter. The nodules were dark and, when observed by transmitted light, they had a globular appearance.

Histopathology: All changes were regressive, as evidenced particularly by autolysis of the exudate within the abscesses and great diminution of the edema and round-cell infiltration. The epithelioid cell zone had been replaced by vacuolated phagocytes and lymphocytes. A small ulcer with granulating base seen in one section probably represented the point through which a submucosal abscess had discharged its contents.

Thirty-Two Days After Infection.—Gross pathology: The intestinal wall was practically normal. There were very few small nodules, none of which contained larvae.

Histopathology: There were no pathologic changes in sections of the ileum and cecum.

II. Observations on Multiple Infections

General Histopathology. — The alterations in this group differed from those encountered in single infections in that there was a diffuse eosinophilic infiltration of the mucosa as well as of the submucosa, in which the eosinophiles were more numerous, particularly within the abscesses (fig. 1D and 2A) and immediate surrounding areas. The early acute reaction with polymorphonuclear leucocytes was less pronounced in this group and the larvae, even quite young ones, which must have penetrated the intestinal wall recently, evoked about them a reaction with epithelioid cells. Giant cells which were not seen in the first group now occurred occasionally about fragments of disintegrating larvae. Lastly, the abscesses contained large numbers of eosinophiles, and presented from the beginning a well developed peripheral barrier of epithelioid cells (figs. 2B and 2C).

Three Days After Administration of Thirty-First Daily Dose of 5,000 Larvae.—Gross pathology: The distal half of the small intestine and the cecum were congested, thickened and covered with nodular lesions of all ages. The inflamed and hemorrhagic condition of the latter is illustrated in figure 2E. The largest nodules were about 4 mm. in diameter and were clearly visible on the serous surface of the intestinal wall. There was some edema of the mesentery of the large intestine.

Histopathology: (See "General Histopathology.")

Forty-Seven Days After Administration of a Series of Seven Doses Totaling 163,000 Larvae.—Gross pathology: The intestinal wall was studded with large nodules 5 mm. in diameter and was greatly thickened. The large intestine showed much greater inflammation than the small one and the nodules were full of pus and fatty material.

Histopathology: In one or two instances the nodules showed a thin capsule of fibroblasts.

One Hundred and Eighty-Two Days After Administration of a Series of Twelve Doses Totaling 260,500 Larvae. — Gross pathology: The healing process had almost obliterated the nodules. The intestinal wall presented thousands of shadowy patches showing the former location of the lesions. There were a few large, caseated nodules containing dead larvae. These nodules could easily be shelled out of the mucosa. There was no active inflammation anywhere in the intestine and no thickening of the mucosa.

Histopathology: The oldest lesions observed measured about 4 mm. across. They were composed of a central cavity filled with purplish debris surrounded by a broad zone of lymphocytes arranged as in solitary lymphoid nodules. Eosinophiles had disappeared from all parts of the intestine except the mucosa. The liver showed nodules 4 mm. across which microscopically represented abscesses with old exudate in the center, an intermediate zone of vacuolated epithelioid cells, and outermost, a broad layer of young fibrous tissue presenting

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE—FIG. 2

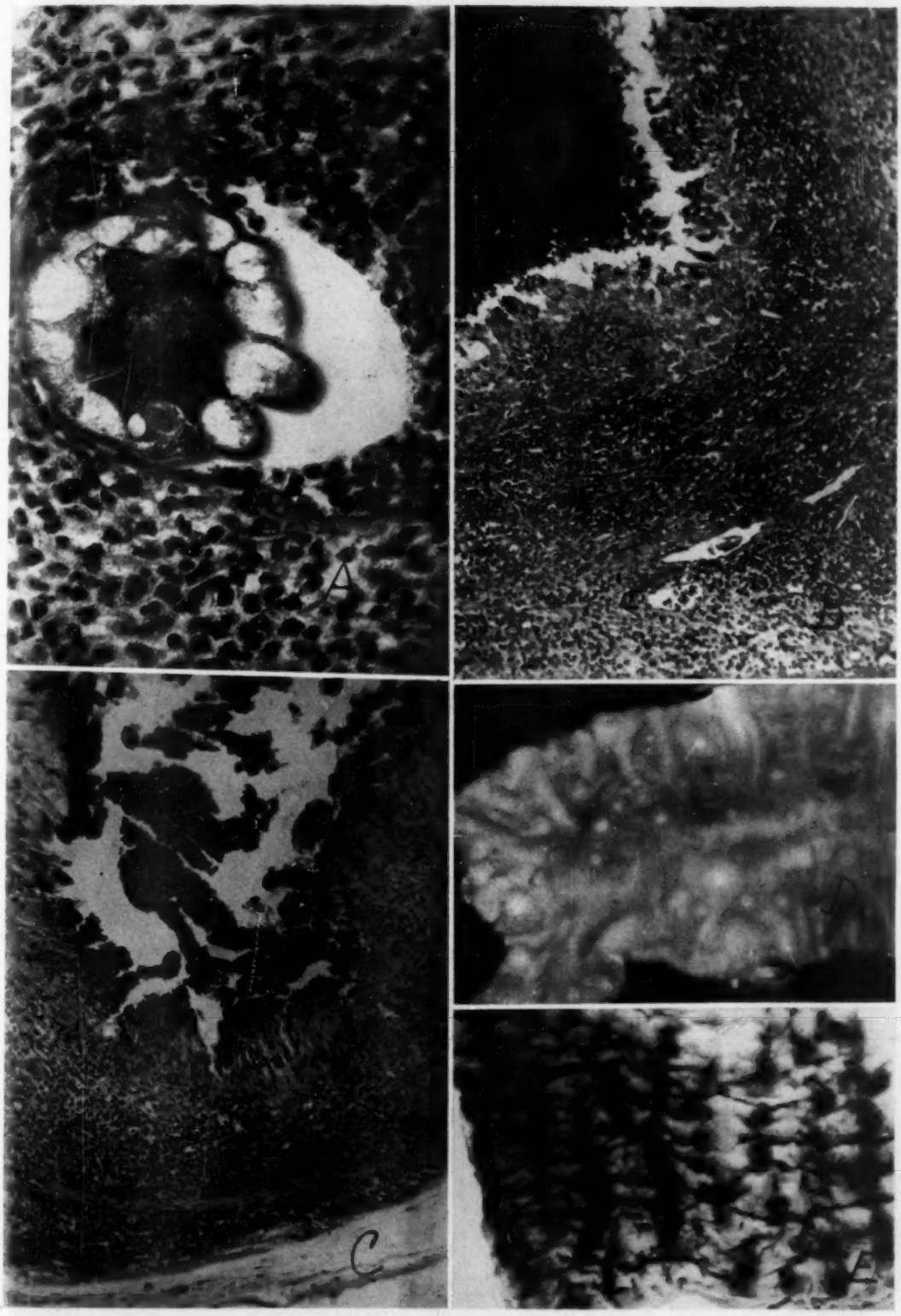
A) Margin of early abscess, showing eosinophiles about dead larva three days after administration of thirty-first daily dose of 5,000 larvae (x 450).

B) Margin of abscess with wall of epithelioid cells (x 100). Same calf as A.

C) Palisading of epithelioid cells about an old abscess 182 days after administration of last dose of larvae; beginning formation of lymphoid tissue at periphery (x 100).

D) Nodules in small intestine. Same calf as A.

E) Portion of cecum, showing thickening of wall and intense inflammation. Same calf as A.


A
B
C
D

small, dense groups of lymphocytes and slight to moderate infiltration with cells of the same type and with a few eosinophiles.

DISCUSSION

Except for the fact that the nodular lesions in the present experimental cases of bovine esophagostomiasis were not limited to the region of the intestine posterior to a point 15 meters back of the abomasum, but were distributed along the whole length of the intestine from the pylorus to the anus, the location, number and gross appearance of the nodules were practically identical with the findings of Marotel.[4] The observations of this investigator, indicating that the size and appearance of the nodular lesions changed with their relative age, was confirmed. In instances of single infections larvae up to and including those observed five days after infection were relatively small and were associated with small, dark nodules approximately 1 mm. in diameter. Larvae observed later than five days and up to about ten days after infection were larger and were associated with nodules measuring between 1 and 2 mm. in diameter and possessing white centers composed of leucocytes. The majority of the lesions 9 to 10 days old and older were ulcerated and contained no larvae, showing that the latter had escaped into the lumen of the intestine. The type of nodule characterized by considerable liquefaction of tissue, which was described by Spindler[5] from the intestines of swine infected with *O. longicaudum*, was observed only in later stages of *O. radiatum* infection when repair of the lesions produced by the larvae had been in progress for about nine days.

Marotel[4] stated that the presence of bacteria belonging to the genus Pasteurella in the abscesses produced by *O. radiatum* larvae, together with the swollen condition of the intestinal lymph nodes, indicated that the bacteria probably contributed to the formation of the nodular lesions. Fourie[6] reported numerous bacteria in lesions produced by *O. columbianum* in sheep. A few years earlier Kinsley[7] found the organism of hemorrhagic septicemia associated with severe esophagostomiasis in steers. Although enlargement of the lymph nodes was observed in the calves studied during the present investigation, bacteria were found in only one small necrotic area in one nodule. This finding seemed to indicate that bacteria played a negligible rôle in producing the pathologic changes taking place in the intestine of calves suffering from experimental esophagostomiasis.

Fourie[6] found that the larvae of *O. columbianum* frequently penetrated the peritoneal cavity of sheep, taking with them bacteria which gave rise to fatal peritonitis in many instances. Such perforation of the intestine of calves by the larvae of *O. radiatum* was not observed in the cases of experimental esophagostomiasis studied. In this connection, it is of interest to note that as early as 1898, Barnes[8] made the observation that Oesophagostomum larvae in the intestinal wall of cattle appeared to be much further removed from the serous surface of the intestine than those in sheep. The greater thickness of the intestinal wall of cattle may account for this observation and for the infrequency with which the larvae perforate the bovine intestine.

Dikmans[9] reported nodules produced by the larvae of *O. radiatum* in the omentum of a calf. The present writers found nodular worm larvae in typical lesions in the liver, spleen and intestinal lymph nodes. This migration of the larvae outside the intestine could easily take place through the blood and lymph vessels of the outer

[5]Spindler, L. A.: Development of the nodular worm *Oesophagostomum longicaudum* in the pig. J. Agr. Res., xlvi (1933), pp. 531-542.

[6]Fourie, P. J. J.: A contribution to the study of the pathology of oesophagostomiasis in sheep. Onderstepoort J. Vet. Sci. & Anim. Ind., vii (1936), pp. 277-347.

[7]Kinsley, A. T.: Esophagostomiasis and hemorrhagic septicemia in steers. Vet. Med., xxvi (1931), p. 506.

[8]Barnes, A. W.: A supposed new disease. Ann. Rpt. Dept. Agr. Queensland, Australia (1897-1898), pp. 88-89.

[9]Dikmans, G.: The infestation of the bovine omentum with nodular worm larvae. Bureau Vet., xii (1936), pp. 1-2.

muscular coat in which numerous larvae were observed.

Mönnig[10] pointed out that the tissue reaction about the larvae of *O. columbianum* was relatively slight in young sheep which had not been infected, while that occurring about larvae in previously infected sheep was relatively intense. Jelen[11] reported that larvae of *O. radiatum* which migrated from the intestinal wall left no trace of their sojourn in the tissues, whereas larvae dying in the wall caused the formation of nodules. This phenomenon also was observed during the present investigation. In calves which received one dose of larvae the intestinal lesions were relatively mild and had disappeared 32 days after infection, while in calves receiving multiple doses of larvae the lesions were much more severe, developed thick fibrous walls and were still visible 47 and 182 days after the last dose of larvae was administered. The early disappearance of lesions from the intestines of calves receiving single doses of larvae is in agreement with the findings reported by Spindler[5] and Veglia[12] in connection with their work on *O. longicaudum* and *O. columbianum*, respectively. The type of nodule seen in calves receiving multiple doses of larvae was similar to that found by Marotel[4] and Descazeaux[13] in calves which had been subjected to more or less continuous daily infection from pastures.

The relationship between the type of cellular reaction about nematode larvae in the tissues of an animal and the development in that animal of an acquired resistance to superinfection with the species of nematode in question was discussed previously by the senior author in connection with observations on this phenomenon during experimental work with the nematode parasite of sheep, *Cooperia curticei*.[14] It is significant that the lesions produced in calves receiving multiple doses of *O. radiatum* larvae were typical of those found to occur about the larvae of other species of nematodes in different species of animals under similar conditions of infection.

Schwartz[15] stated that the severity of the lesions produced in swine by esophagostomes was closely related to the species involved. He reported that *O. dentatum* produced small, uninflamed nodules, whereas *O. longicaudum* gave rise to nodules which were larger and more intensely inflamed. Whether similar differences may occur in lesions produced in cattle by different species of esophagostomes, or whether such differences, if they do occur, may be accounted for on the basis of the type of infection (single or multiple) to which the animal was subjected, still remains to be determined experimentally.

A slight inflammation of the intestinal mucosa was noted in calves harboring infections composed only of adult nodular worms, but there was no demonstrable tissue pathology. This finding was in keeping with that of Wetzel,[16] who found that adult esophagostomes did not cause gross injury to the tissues, but fed on the products of the irritated and inflamed mucosa. This observation also indicated that the intestinal pathology of *O. radiatum* was confined to the penetration of the larvae into the mucosa and to the tissue destruction and inflammation resulting therefrom.

Gordon[17] and Veglia[12] have reported cases of intussusception associated with esophagostomiasis in sheep. Mönnig,[18] however, indicated that this condition was due to causes associated with changes in

[10]Mönnig, H. O.: Veterinary Helminthology and Entomology; the Diseases of Domesticated Animals Caused by Helminths and Arthropod Parasites (London), 402 pp.

[11]Jelen, G. D.: Studies on the nematode, *Oesophagostomum radiatum*. Vet. Alumni Quart., Ohio State Univ., xxi (1938), pp. 89-97.

[12]Veglia, F.: Oesophagostomiasis in sheep. Preliminary note. Union S. Africa, Dept. Agr., Dir. Vet. Ed. & Res., 13th & 14th Rpts. (1928), pp. 755-797.

[13]Descazeaux, J.: L'entequé chilienne et ses rapports avec l'oesophagostomose nodulaire. Rev. Chil. de Hist. Nat. Sant. de Chile, xxx (1926), pp. 106-112.

[14]Andrews, J. S.: Life history of the nematode *Cooperia curticei*, and development of resistance in sheep. J. Agr. Res., lviii (1939), pp. 771-786.

[15]Schwartz, B.: Nodular worm infestation of domestic swine. Vet. Med., xxvi (1931), pp. 411-415.

[16]Wetzel, R.: Zur Ernahrung und pathogenen Wirkung des Oesophagostomum. Deut. Tierärztl. Wochnschr., xlii (1934), pp. 602-603.

[17]Gordon, H. McL.: Intussusception associated with oesophagostomiasis. Australian Vet. J., xiii (1937), p. 126.

[18]Mönnig, H. O.: The cause of nodular enteritis in cattle. Onderstepoört J. Vet. Sci. & Ani. Ind., i (1333), pp. 63-65.

diet which were entirely removed from infection with intestinal parasites. In the present experiment intussusception was not observed nor was it seen in the field cases of nodular worm disease examined.

SUMMARY

The data reported in this paper show that the nodular worm, *Oesophagostomum radiatum*, is capable of producing severe pathologic changes in the intestines of previously healthy calves which were artificially infected with large numbers of the infective larvae of this nematode. The larvae were found to penetrate the intestinal wall anywhere from the pylorus to the anus, but the majority of them entered the of the ileum, cecum and colon. Wherever the larvae penetrated, they were found in all parts of the intestinal wall from the mucosa to the outer muscular coat, inclusive, but the principal inflammatory changes were seen in the submucosa.

A total of 3,598 to 20,000 nodular lesions, distributed as indicated previously, were found in the intestines of calves autopsied either during the clinical period of the disease or shortly thereafter. These lesions varied in size and appearance according to their age and to the type of infection (single or multiple) to which the experimental calf had been subjected. The nodules associated with young larvae (up to and including those observed 5 days after infection) were about 1 mm. in diameter and dark. The lesions associated with older larvae (from five to about ten days after infection) were from 1 to 2 mm. in diameter and exhibited a white center composed chiefly of leucocytes. Many of the nodules observed ten days or more after infection presented an orifice connecting the interior of the nodule with the lumen of the intestine, indicating that the larva had migrated from the nodule.

The lesions in calves infected with single doses of larvae were found to be relatively mild and were made up of polymorphonuclear leucocytes. In this group the pathologic process reached its height about ten days after infection and the majority of the lesions had disappeared from the intestine 22 days later, or 32 days after infection. The lesions in calves receiving multiple doses of larvae were much more severe, were made up of eosinophiles, contained giant cells and possessed epithelioid capsules which in some instances were surrounded by fibroblasts. They were still visible 47 and 182 days after the last dose of larvae was administered and many of them still contained nematodes. The intestines of the calves receiving multiple doses of larvae also showed much more severe inflammation and edema than those of calves receiving single doses of larvae. The intestine of the calf autopsied 182 days after the completion of infection showed no active inflammation and the great majority of the nodules had disappeared, leaving only faint shadows marking their former position.

The differences between the lesions produced in these two groups of calves were similar to those previously observed by the senior author and by other investigators during studies on the relationship between the type of cellular reaction about other species of nematode larvae in the tissues of different hosts and the development in these animals of acquired resistance to superinfection with the species of nematode involved.

Bacteria were apparently of little importance as agents of secondary infection in the cases of experimental esophagostomiasis studied. This finding was contrary to the observations of other investigators who have reported that bacteria were apparently involved in esophagostomiasis of both sheep and cattle. The importance of bacteria in esophagostomiasis of sheep appears to depend upon the frequent perforation of the intestinal wall by the larvae of *O. columbianum*, which thus introduce the organisms into the peritoneal cavity where they give rise to fatal peritonitis. Perforation of the intestine of calves by the larvae of *O. radiatum* does not appear to occur as often as it does in sheep, due perhaps to the relatively greater thickness of the wall of the bovine intestine.

No demonstrable pathology was observed in calves harboring infections composed of adult *O. radiatum*, thus indicating that the

pathologic changes in the intestine of calves suffering from esophagostomiasis were apparently confined to the tissue destruction and inflammation arising from the invasion of the intestinal wall by the nodular worm larvae.

ACKNOWLEDGMENTS

The authors express their appreciation to Dr. Enrique Koppisch, head of the Department of Pathology of the School of Tropical Medicine, for his coöperation in the histologic examination of tissues from the experimental animals and to the Puerto Rico Reconstruction Administration for supplying funds for necessary aid. They also thank Messrs. Fonalledas and Nevares for donating the experimental animals used in this investigation.

Observations on the Pathology of Dairy Calves on Low Vitamin A Diets*

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ABNORMAL, PHYSIOLOGIC and tissue changes due to a low intake of carotene have been reported in the dairy calf.

REVIEW OF LITERATURE

Moore, Huffman and Duncan[1] reported on a blindness in calves of nutritional origin associated with constriction of the optic foramen. Moore[2] reported that calves fed low carotene rations developed nyctalopia, papilledema and a permanent blindness due to constriction of the optic nerve. Guilbert and Hart[3] established the relationship of night blindness to vitamin A deficiency in the bovine species. A degeneration of the germinal epithelium of the testes, absence of spermatozoa in the epididymus and changes in the pituitary gland were reported by Sutton, Krauss and Hansard.[4] Moore and Sykes[5] showed that increased cerebrospinal pressure was associated with vitamin A-deficient diets in the bovine species. This observation was found to correlate with the papilledema occurring in calves. Langham, Sholl and Hallman[6] described a degeneration of the tubular epithelium, numerous lesions of cellular infiltration and proliferation and fibrosis in the kidney of cattle deficient in vitamin A.

From the above, one would conclude that certain physiologic tissue changes are associated with a low intake of vitamin A.

MATERIALS AND METHODS

During the past 2½ years, 25 calves from the dairy experimental herd were autopsied in the course of vitamin A studies.

These 25 cases were studied grossly and microscopically and are divided into three different groups. The animals of each group received different amounts of vitamin A in the form of carotene. Group 1 consisted of 13 calves which had received the lowest level of carotene, with an average daily intake of 6.7 micrograms per calf. Group 2 consisted of eight calves which had received a low to medium intake of carotene, with an average of 15.8 micrograms per calf daily. Group 3 consisted of four calves which had received the highest levels, or an average daily intake of 19.7 micrograms. Detailed data concerning the blood carotene, etc., are given elsewhere.[7]

GROSS PATHOLOGY

Enteritis was observed in eleven of the cases in group 1, five of the seven in group

*Paper No. 1026, journal series, Pennsylvania Agricultural Experiment Station.

[1]Moore, L. A., Huffman, C. F., and Duncan, C. W.: Blindness in cattle associated with a constriction of the optic nerve and probably of nutritional origin. J. Nutri., ix (1935), p. 533.

[2]Moore, L. A.: Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema and nyctalopia in calves. J. Nutri., xvii (1939).

[3]Guilbert, H. R., and Hart, G. H.: Minimum vitamin A requirements with particular reference to cattle. J. Nutri., x (1935), p. 409.

[4]Sutton, T. S., Krauss, W. E., and Hansard, S. L.: The effects of vitamin A deficiency on the young male bovine. Abst., J. Dairy Sci., xxiii (June 1940); Sutton, T. S., and Brief, B. J.: Anatomical and physiological changes in the pituitary glands of vitamin A deficient rats. Bul. 610, Ohio Agr. Exp. Sta.

[5]Moore, L. A., and Sykes, J. F.: Cerebrospinal fluid pressure and vitamin A deficiency. J. Physiol., cxxx, p. 684.

[6]Langham, R. F., Sholl, L. B., and Hallman, E. T.: The pathology of the bovine kidney in vitamin A deficiency. Abst., J.A.V.M.A., xcvii (Nov. 1940), pp. 542-543.

[7]Unpublished data from the Dept. of Dairy Husbandry, Pennsylvania State College.

2 and two of the four in group 3. The prevalence and severity of the enteritis appeared greatest in the calves on the lowest levels of vitamin A. Diarrhea, which was observed periodically during the course of the study, became more severe toward the end of the experiment. As a rule, the chyme consisted of some food and large quantities of mucus. Upon section, the mucosa was found to be swollen and thickened and the mesenteric lymph nodes were swollen and edematous.

The gross lesions in the kidneys varied in individual cases. The predominate lesions were numerous white foci ranging in size from pin point to 1 mm. The capsules stripped easily from the cortex with the exception of two atypical cases in group 1. These two were typical of "white-spotted kidney" in cattle. Upon section some of the pin-point foci were found to extend into the cortex while others noted were deeper in the cortical substance. With the exception of the two cases mentioned above, lesions were found in ten of the cases in group 1, one of the eight in group 2 and one of the four in group 3.

Grossly the liver lesions were not numerous and they varied considerably. A mottling of the parenchyma of the liver suggesting necrosis was the predominate change. However, one case in group 2 had multiple abscesses of long duration, while two in group 1 suggesting fibrosis of the liver were the exceptions. In three cases, the gall bladder contained considerable dark, thick bile. Eight of the cases in group 1 showed liver lesions, two in group 2 and one in group 3.

A typical bronchopneumonia was observed in 4 of the 25 cases. Two of them were on the lowest level of vitamin A. The others were on the next to the lowest level, or group 2.

HISTOPATHOLOGY

Sections for microscopic study were fixed in both Zenker's solution and formalin. In a few cases Bouin's solution was used. Sections were stained with Heidenhain's aniline blue, Verhoeff's elastic tissue stain, sudan IV for fat and eosin hematoxylin.

The most conspicuous lesions were observed in the kidney, liver, intestine and testicle.

Degeneration of the epithelium of the proximal convoluted tubules existed in all of the groups, but it was most extensive in group 1. The degeneration and necrosis in Henle's loop and the distal convoluted tubules were not as conspicuous as in the other parts of the tubules. Two cases in group 1 showed some evidence of fatty degeneration of the epithelium in the proximal convoluted tubule. Hyaline-like material in the tubular epithelium was present in a number of cases. This was especially noticeable in those areas where considerable atrophy and degeneration of the tubules had taken place. Those areas which contained grayish-white pin-point foci were characterized by accumulations of lymphocytes and macrophages (figs. 1 and 2). In other areas there was a marked proliferation of fibroblasts and considerable fibrosis (fig. 3). In some of these areas a complete degeneration of the tubules had occurred. In the more conspicuous lesions there was a connective tissue thickening of

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE—FIGS. 1-8

1) Section through the cortex of the kidney, showing one of the pin-point lesions noted in gross. Note the loss of the tubules and accumulation of lymphocytes and macrophages (x 85).

2) Section of the cortex, showing atrophy of the tubules and a marked cellular infiltration (x 85).

3) Section of the cortex, showing atrophy of the tubules and early fibrosis (x 85).

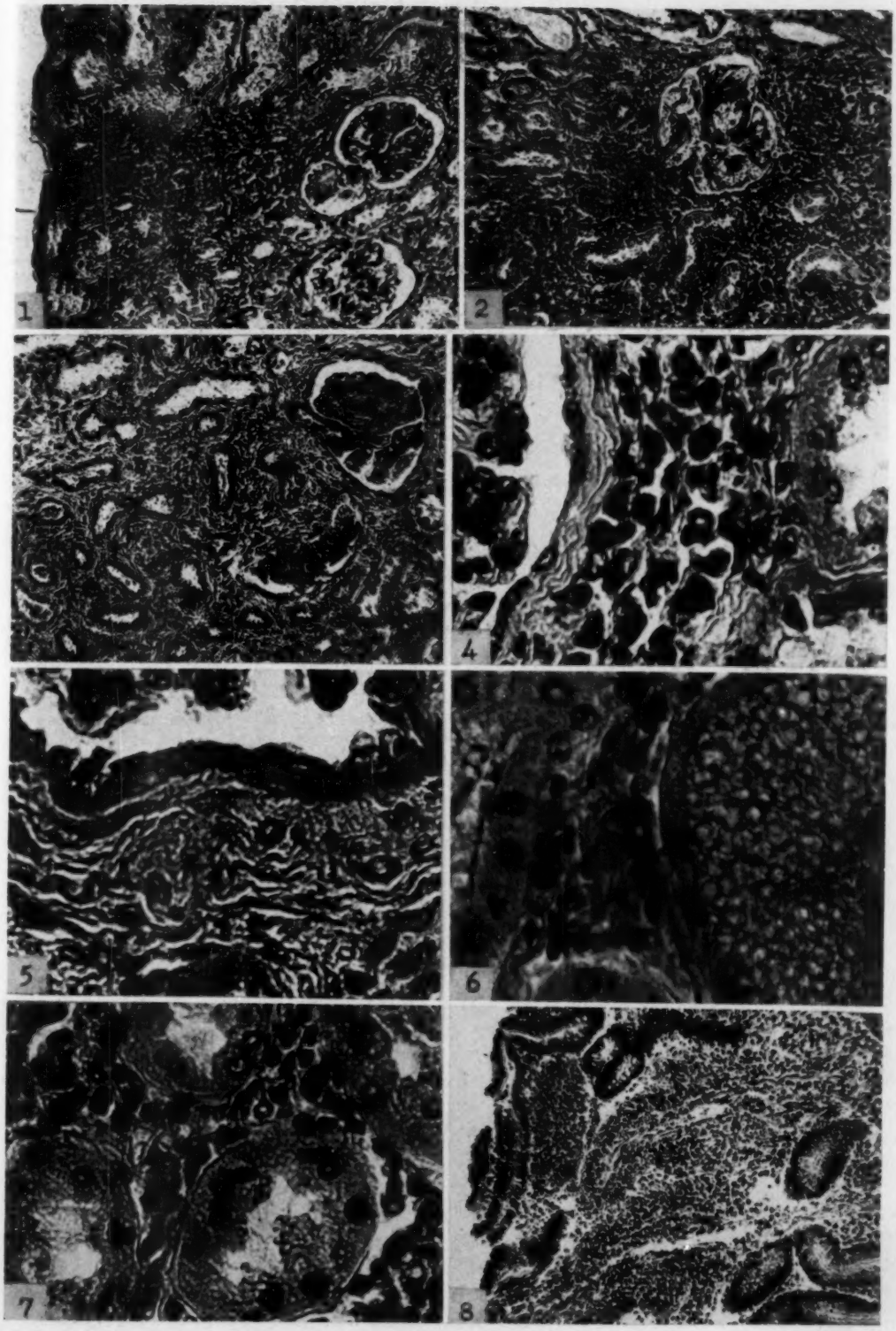
4) Section at the periphery of the renal corpuscle, showing early fibrosis of Bowman's capsule (x 455).

5) Section, showing marked thickening and hyalinization of Bowman's capsule (x 450).

6) Section of the cortex, showing recent interstitial infiltration of lymphocytes and macrophages with marked hemorrhage into the tubules (x 465).

7) Section, showing an infiltration of lymphocytes and macrophages about the tubules with early degeneration of the tubular epithelium (x 450).

8) Section of small intestine, showing necrosis and inflammatory changes with some thickening of the mucosa (x 85).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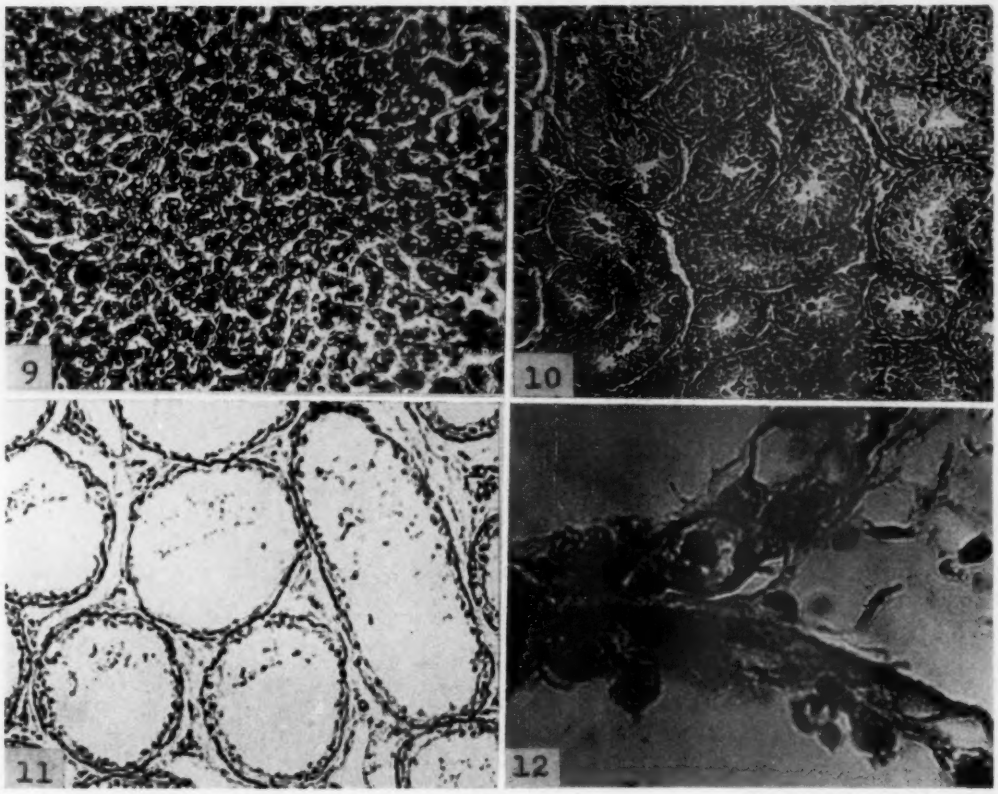

FIGS. 9-12

9) Section of liver, showing degeneration of the cells (x 70).
10) Section of normal, developing testicle (x 115).
11) Section of testicle, showing extensive degeneration of the germinal epithelium (x 120).
12) Section of testicle, showing degenerated germinal epithelial cells (x 475).

Bowman's capsule (figs. 4 and 5) accompanied by some atrophy of the glomerular tufts. Considerable fibrosis was observed in the tissues adjacent to the atrophied and thickened renal corpuscle. With but two exceptions the cases showing the most fibrosis and degenerative changes were in group 1. In these two cases there was a moderate degeneration of the tubular epithelium and early cellular infiltration with very little proliferation of fibroblasts. In three cases of group 1, considerable hemorrhage into the tubules was noted (fig. 6). The lesions in which there was an infiltration of lymphocytes and macrophages about the tubules were usually accompanied by an early degeneration of the tubular epithelium (fig. 7).

The intestinal mucosa of most of the calves showed a varying degree of morbidity. Most of the lesions consisted of a mild, somewhat hyperplastic enteritis beginning in the upper small intestine and extending to the ileocecal valve. Considerable necrosis and desquamation of the epithelium were present in some cases (fig. 8). Sections removed at 4- to 6-ft. intervals beginning just below the pylorus seemed to indicate an increase in the severity of the lesions in passing toward the ileocecal valve. Some necrosis of the epithelium and infiltration of the stroma of the glands with lymphocytes and macrophages were noted, accompanied in some areas by the proliferation of fibroblasts. A large number of eosinophiles were present in the mucosa. With the exception of three cases that showed an acute diarrhea clinically, there was no evidence of desquamation and ulceration of the mucosa.

Microscopically the most consistent lesions seen in the liver were cloudy swelling and necrosis, and in five cases of group 1 there was some fatty degeneration (fig. 9). In the cases where necrosis of the liver cells had taken place, there were small foci of macrophages and lymphocytes.

The lesions in the testicle consisted of a degeneration and necrosis of the germinal epithelium lining the tubules (figs. 10 and 11). No marked interstitial changes were observed. There were sporadic attempts at spermatogenesis in these areas. Areas in which the epithelium was not entirely destroyed showed many pyknotic and degenerate nuclei (fig. 12).

Metaplasia of the epithelium was noted to some extent in the calices of the kidney, and in two cases this was observed to a moderate degree in the trachea. These changes were not as conspicuous in the calves on the lower levels as one would expect.

DISCUSSION

Although some variations occurred in the microscopic pathology of the tissues studied, certain degenerative and inflammatory lesions occurred consistently. With the exception of the lesions observed in the liver tissue, changes were more extensive in those cases on the lowest levels of carotene. The duration, severity and extent of the lesions and symptoms in the intestinal tract were manifested in the liver and kidney. This was especially true as regards the kidney lesions. Although the enteritis in most cases was a mild, somewhat hyperplastic lesion, a correlation appeared to exist between the extent of the intestinal pathology and the pathology of the other tissues. The liver lesions were the most variable. In three of the cases on the lowest levels of vitamin A, there were no marked liver lesions although considerable diarrhea, enteritis and conspicuous kidney lesions existed.

The degenerative changes in the testicle did not exist in the cases on the higher levels of vitamin A. A number of the cases were autopsied too young to determine the effect on the number of spermatozoa, but degenerative changes were observed in the germinal epithelium which probably would have affected spermatogenesis in 9 of the 13 cases in group 1.

In considering all the lesions found on the three levels of carotene, it is obvious that the more marked lesions occurred in the lowest level of vitamin A. This difference in the various groups was noted especially in the pathology of the kidney.

SUMMARY

1) Twenty-five calves fed three different suboptimal levels of carotene were studied grossly and microscopically for pathologic changes.

2) Degenerative and inflammatory changes were observed in the kidneys of calves on the lower levels of vitamin A.

3) An enteritis with some thickening of the mucosa accompanied by a persistent diarrhea was observed.

4) Degeneration and necrosis were present to a variable extent in the liver.

5) Degenerative changes also were observed in the testicle. These probably inhibited normal spermatogenesis.

6) Metaplasia of the epithelial tissues was not consistently observed.

7) A correlation appeared to exist between the lesions in the intestines and in the kidneys, both in extent and severity. In a limited number of cases the liver could be included in this relationship.

8) In general, the lesions observed were more conspicuous in the calves which had received the lowest levels of vitamin A.

A Method of Determining the Reproductive Efficiency of Cattle*

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THIS THESIS DISCUSSES the objects of statistical treatment of data relating to reproduction in cattle. It lays the foundation for the study of certain phases of genetics.

It gives a method of keeping records and annotates the successive steps taken.

It shows how to arrange the breeding histories of individuals so that either groups or individuals can be studied separately or collectively.

It presents tables supplemented by charts and graphs for statistical interpretation.

The general principles advanced by Mead[1] and Birch[2] are incorporated in exhibit A. Birch pointed out the necessity for such a plan in the following statement: "The calculation of reproductive efficiency must be centered upon the months of normal pregnancy." The method used in the preparation of exhibit A is similar to that used by Birch. Fortunately, both Mead and Birch made their methods flexible enough to allow drastic modifications without deviating from the fundamental principles.

Exhibit A and associated tables show the reproductive efficiency of a herd from 1923 to 1933. Exhibit A is an imaginary herd intended to illustrate the methods of analysis.

This paper also discusses different methods of calculating reproductive efficiency and evaluates their merits.

It has been prepared in such a way that it can be understood by the laymen as well as the scientific breeder.

An analysis of this type must be made to determine the reproductive efficiency of the herd, to learn from the experience of the past and to profit from the knowledge of our failures and successes. To trust to memory is a dangerous policy. Written records are not always correct but, at least, they do not change.

Records become voluminous and lose value unless they are arranged and condensed so that they are understandable. Assembling certain facts from the records is like winnowing the grain from the straw. The important things gradually take form and the unimportant ones fade into the background.

Frequently, apparently unimportant items multiply until they become dominant factors. The object of statistics is to relegate unimportant facts and preserve and emphasize relevant facts contained in the data.

Mathematical application to a problem encourages deductions that are based upon facts and are not influenced by emotions. When statements are made about any subject, the most important question to set forth is, "Upon what is the opinion based, and what methods were used in arriving at the conclusion?"

The first glance at the problem of determining the reproductive efficiency of a breeding herd gives the impression that it is a simple matter and that the only requirement is to count the cows and calves and compute the ratio in terms of percentage. Unfortunately, such a method is not satisfactory and is misleading. At the time, it gives some idea of what is taking place, but it only roughly estimates reproductive efficiency.

SOURCES OF DATA

1) Daily records are kept by the foreman, the herdsman or the manager. Observations are recorded in pocket notebooks or field books.

2) The daily reports (form A) are made from the daily records.

*From the Wyoming Hereford Ranch Trust.

[1]Mead, S. W.: A diagrammatic method of presenting the history of reproduction in a dairy herd. J. Dairy Sci., xxi (June 1938), pp 283-287.

[2]Birch, R. R., *et al.*: The handling of Bang's disease in the field. Ann. Rpt., New York State Vet. Coll. (1932-1933).

3) The cards (form B) are compiled from the daily reports.

4) Exhibit A was compiled from the cards.

Form A.—Information gathered during the day is transferred from the field book to the daily report sheet. This insures consistent, orderly recording. The daily report sheets assembled in a folder make up the monthly record.

Form B.—There are many advantages to the card system. It affords a record of daily and monthly facts that relate to the problem of reproduction.

Cards can be filed in an orderly fashion. Daily and monthly recordings should be brief. This encourages accuracy and conserves time. Cards are valuable in determining the continuity of an individual's record. However, in order to be of greater value the records of individuals must be assembled in such a manner that groups, as well as individuals, can be studied.

Form C.—Special data, such as the veterinarian's report, can be stapled to the individual cards. These data are of great value, but they must be considered as a factor separate from the first analysis.

EXHIBIT A

1) Exhibit A presents, in diagrammatic form, the information collected and recorded in the field books, monthly reports and cards.

2) It makes possible a conception of the amount of data at hand.

3) Its presentational character allows one to make rough estimates of the reproductive efficiency of the herd over a period of months or years.

4) It permits the study of individuals as well as groups.

5) Groups can be picked at random. For example, it is possible to select the records of reactors to Bang's disease, or cows of a certain age, and analyze them separately or in groups.

6) It shows the effects upon reproduction of such factors as disease, nutrition, season, weather and age.

7) It permits the division of the gestation period into nine parts, so that any part of the period can be assigned to any chosen time.

8) Within certain limits it permits the determination of the potency of the bull as well as the fertility of the cow.

9) It calls attention to the degree of success or failure.

10) It lays the foundation for statistical analysis.

11) Each bull is given a code letter or number.

12) The cows are identified by horn brand as indicated in the first column of this exhibit.

13) A double line in exhibit A represents a nine-month period of gestation. It is always just nine months long. It can be divided at any monthly point. It includes the month of service, but does not include the month of parturition. This is not always fair in individual cases since the service may have been a few days from the end of the month, and parturition may have occurred at the end of a month that is not indicated by the double line. The law of averages probably corrects the error.

STATISTICAL ANALYSIS OF EXHIBIT A

The general purpose of statistical treatment is to reduce large groups of numerical data to a few figures which, in conjunction with a set of fundamental principles, adequately represent the original data. The statistical machinery in no way changes the adequacy of the data; hence, no statistical result is more reliable than the measurements from which it is derived.

Homogeneity of data is described by White[3] as follows:

> Homogeneity in statistics refers to data of the same kind. Apples and potatoes can not be added. The greater the degree of likeness, or homogeneity, the more reliable will be the results of statistical analysis. Homogeneity is a matter of degree, and the highest degree is to be desired. Any degree of heterogeneity introduces extraneous factors which have to be considered or neglected. If they are neglected, the validity of the results of the study is questionable to the extent of the influence of the extraneous factors.

The truth of this statement became apparent at the beginning of the analysis of

data relating to the so-called calf crop. Lack of ability to select a single factor and adhere to it caused confusion.

Extraneous factors, such as number of services, voluntary or involuntary estrual periods, disease, unproductive months of pregnancy, and rest periods must be considered separately.

There is an inclination to lay too much stress upon the average, or percentage, of reproductive efficiency. All that can be gleaned from the average percentage is that the efficiency is high or low. It does not point to the causes of either a high or a low figure.

METHOD OF CALCULATION

The method of arriving at the reproductive efficiency of the herd is based upon the assumption that when a cow has nine months of normal pregnancy during a twelve-month period, she should be given a rating of 100 per cent. This is called "par." This standard of measurement appears to be the one that best fits the problem.

It is fundamental that the calculation must be centered upon the months of normal pregnancy (M). The period of gestation in the cow is nine months. If the total number of months of normal pregnancy is divided by 9, the result will represent the normal calf equivalent (C_q), *i.e.*,

$$\frac{M}{9} = C_q$$

The next step is to obtain the number of cow years (C_r).

A cow year—the basis of calculation—is twelve months. When, however, a cow is in the herd for nine months or more, she is considered to be in the twelve-month class. The cow year can not start with the date of service and end with any chosen time of observation. It must be a multiple of twelve, not a fraction of twelve.

The reproductive efficiency (R) is determined by dividing the normal calf equivalent (C_q) by cow years (C_r) and multiplying by 100, *i.e.*,

$$\frac{C_q}{C_r} \times 100 = R$$

Confusion may arise in using this method of calculation because of the attempt to introduce into the formula closely related but extraneous factors, such as number of services, unproductive eligible months and rest periods. The fact that three months of "leeway" is given out of every twelve months allows such problems to enter the picture without changing the fundamental premise. Therefore, in making the calculation upon the nine-month—twelve-month—basis, one must ignore all related factors and confine the interpretation to the premise as stated.

TABLE I

Table I shows the months of normal pregnancy produced by the individuals during a twelve-month period. The data obtained from exhibit A include the years 1923 through 1932. The number of cow years is obtained by counting the number of cows in each year.

Table I does not demonstrate the character of the causes that are responsible for success or failure; it simply points out the degree to which success or failure occurs.

GRAPH I

The solid line shows the reproductive efficiency of the entire herd. It is based upon the premise that nine months of pregnancy during a twelve-month period is equivalent to 100 per cent reproductive efficiency. It includes all animals regardless of the length of time they were in the breeding herd during each year.

The dotted line shows the reproductive efficiency of the herd after omitting the cows that were in the herd less than nine months during each year.

Measures in central-tendencies values are useful for concise description of the data, but the tendency of the data to scatter below and above the central value is as noticeable as is concentration. An adequate description of a frequency distribution requires knowledge of both scatter and concentration.

TABLE II

This table shows the frequency of distribution of cows according to the months

DAILY REPORT

Reported by John Jones Date July 15, 1941

CALVES DROPPED			
Cow No.	Sex	Tattoo	Sire
162-22	F	5-37	B
305-22	M	7-37	B

COWS BRED	
Cow No.	Bull used
183-21	A
207-21	B
949-21	B

ABORTIONS

DEATHS

Date 7/14/32 Tattoo 48-21

Appearance and condition Good

Mammary gland Normal

Vulva Normal Discharge None

Vagina Normal

Cervix Sealed - small

Uterus R. H. Slightly enlarged.

Uterus L. H. Small

Tubes R. Normal L. Normal

Ovary R. Corpus luteum

Ovary L. Small normal

Microscopic study of exudates None

Diagnosis With calf - 60 days.

Treatment None

Prognosis Favorable

Left: Form A.
Below: Form B.
Right: Form C.

Name_____ Date of Birth_____ Tattoo & Horn Brand_____

Sex | Date of Vaccination: | Date of Purchase:

Date Bred	Name of Bull	Date Calved	Sex of Calf	Name of Calf	Tattoo & Horn B.	Bang Tests

TABLE I

Statistical Analysis of Information Contained in Exhibit "A"

Year	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932
Months Normal Preg-nancy	5 5 8 7 6 7 0 0 6 7 7 7 9	6 6 0 3 8 4 6 0 6 7 9 6 6 6 3 2 8 7 6	7 10 8 6 10 9 6 8 4 8 10 9 10 3 9 6 9 7 10 9	7 7 9 3 8 5 6 7 10 11 9 8 10 7 3 9 8 9 7 10	9 9 9 8 0 11 6 10 8 3 6 3 10 3 8 5 9 9 8 4 8	1 0 10 5 10 10 0 4 9 11 9 9 5 9 7 6 8 4 3 8 10 9 9	8 8 9 4 9 10 4 0 9 9 4 9 4 6 5 3 9 6 6 9 8 9 9	9 1 3 6 6 9 10 5 9 5 10 11 6 9 6 7 9 4 9 10 10 8 10	4 5 9 0 6 3 10 9 10 8 9 11 10 9 9 9 7 8 10 11 9 9 6 6	9 10 9 5 9 6 10 5 8 9 9 10 9 9 9 9 5 8 9 3 3 3 10 5
Total Cow Years	13	19	20	20	21	23	23	23	25	25
Total Mo. Norml. Preg.	74	99	158	153	146	156	157	172	196	190
Nrml. Calf Equiv.	8.25	11.0	17.6	17.0	16.40	17.35	17.44	19.11	21.77	21.11
% Rep. Eff.	64	58	88	85	78	75	76	83	87	84

GRAPH I

Data Taken From Table I — Shown Above

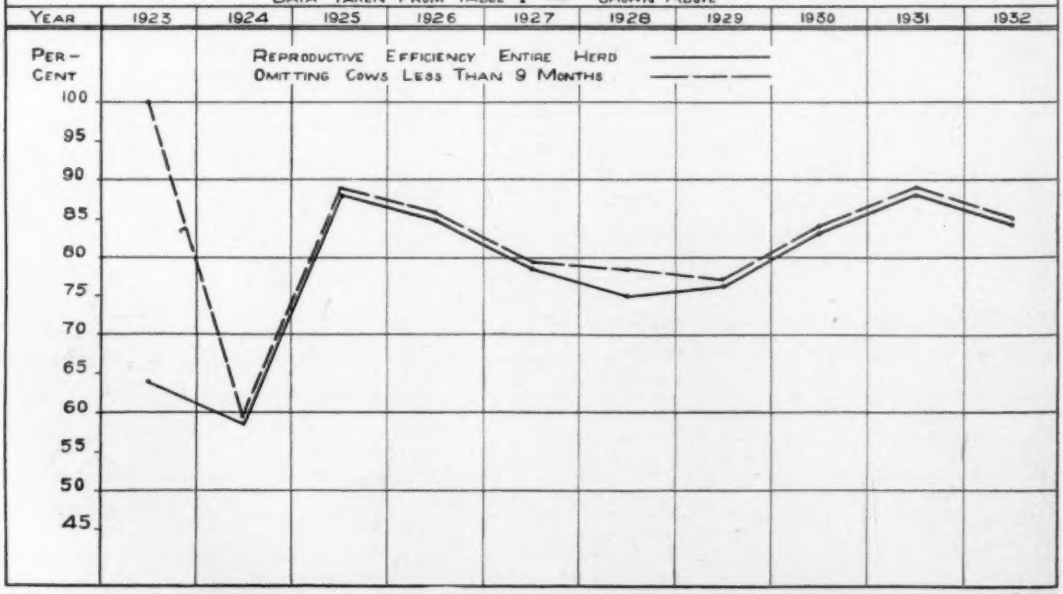


TABLE II

FREQUENCY DISTRIBUTION OF THE MONTHS OF NORMAL PREGNANCY

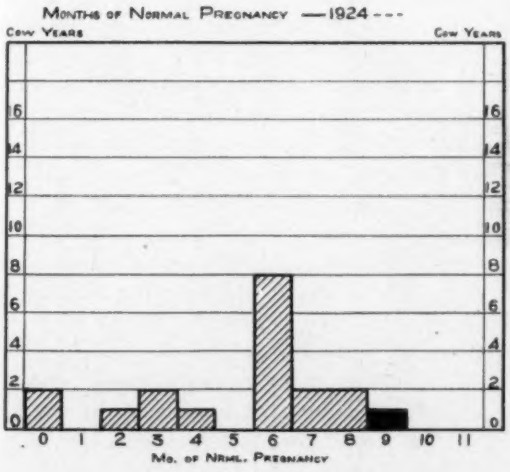
X	1923		1924		1925		1926		1927		1928		1929		1930		1931		1932		1933	
	F	FX	F	FX	F	FX	F	FX	F	FX	F	FX	F	FX	F	FX	F	FX	F	FX	F	FX
0	2	0	2	0	0	0	0	0	1	0	2	0	1	0	0	0	1	0	0	0	3	0
1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0
2	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	2	6	1	3	2	6	3	9	1	3	1	3	1	3	1	3	3	9	5	15
4	0	0	1	4	1	4	0	0	1	4	2	8	4	16	1	4	1	4	0	0	2	8
5	2	10	0	0	0	0	1	5	1	5	2	10	1	5	2	10	1	5	4	20	0	0
6	2	12	8	48	3	18	1	6	2	12	1	6	3	18	1	6	0	0	0	0	0	0
7	5	35	2	14	2	14	5	35	0	0	1	7	0	0	1	7	1	7	0	0	0	0
8	1	8	2	16	3	24	3	24	5	40	2	16	3	24	1	8	2	16	2	16	2	16
9	1	9	1	9	5	45	4	36	5	45	6	54	9	81	6	54	9	81	11	99	6	54
10	0	0	0	0	5	50	3	30	2	20	4	40	1	10	5	50	4	40	4	40	3	30
11	0	0	0	0	0	0	1	11	1	11	1	11	0	0	1	11	2	22	0	0	2	22
Sum F	13		19		20		20		21		23		23		23		25		25		23	
Sum FX		74		99		158		153		146		156		157		172		196		190		145

X = MONTHS NRML. PREGNANCY
F = NUMBER OF COWS
FX = MONTHS NRML. PREG. MULTIPLIED BY NO. OF COWS

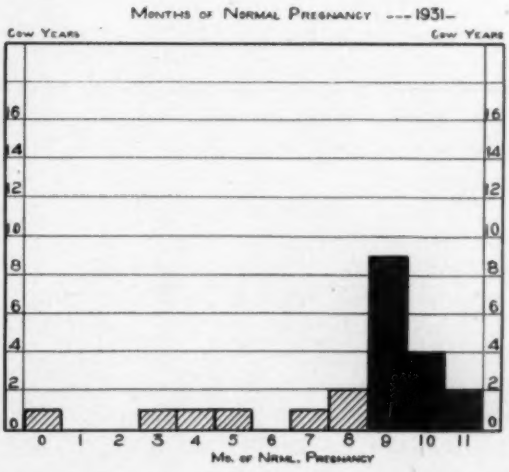
GRAND TOTAL F = 266
GRAND TOTAL FX = 1755

GRAPHS

II



III



of normal pregnancy produced. The data were obtained from table I. The number of months or class interval is arranged successively from zero to eleven, in the X column.

The F columns indicate the frequency with which the cows produced the months of normal pregnancy shown in the class interval.

The Fx columns indicate the total months of normal pregnancy produced.

The product of the number of cow years

and the frequency show the months of normal pregnancy for that group. For example, in 1923, exhibit A, there are five cows, each giving a frequency of seven months of normal pregnancy during a twelve-month period. Together they produce 35 months of normal pregnancy. The sum of all of the products gives the total months of normal pregnancy for all cows during that year.

The objects of the frequency distribution table, and graph, are to call attention to proportionate numbers of cows that have produced par (nine months of normal pregnancy during a twelve-month period) and to compare one year with another.

GRAPH II

This graph is derived from the data of table II. It shows the number of months of normal pregnancy produced and the number of cows in each class as indicated at the margin.

The classes are from one to eleven months as indicated at the bottom of the page.

The solid bars show the number of cows that produced nine, ten and eleven months of normal pregnancy during the year. According to method 1, these groups have produced par or above par. Nine months is accepted as par.

The cross-hatched bars indicate the number of cows that produced zero to eight months of normal pregnancy. This group is below par.

The chart shows the relation of the number of cows which reached par to those which did not.

Table II and graph II on frequency distribution show concentration about the nine-month area, but they also call attention to the dispersion away from the nine-month area. All of those cows which fail to reach the nine-month figure (par), fail according to the nearness to that area. Therefore, they are subjects for investigation.

Scatter is usually referred to in statistics as dispersion or variation. Deviations from the central value, or from par, may be due to a variety of causes. It has been stated that analysis points out the degree to which dispersion exists. Further study attacks the more vital problem of the causes of dispersion from par. For example, individual cows may have been in the herd less than nine months; therefore, they can not be included fairly. On the other hand, certain groups may have been bred to bulls of low fertility, thereby prolonging the eligible unproductive months and forcing the months of normal pregnancy below par.

A common cause of deviation from the normal is infertility on the part of the cow. Too long a rest period is often responsible for infringement upon months of normal pregnancy during a fixed period. Infectious diseases, such as brucellosis and trichomoniasis, take their toll of productive months. Finally, the removal of cows from the herd prior to the time of parturition places them in the doubtful class. They should not be considered a part of the breeding herd.

One could go on indefinitely pointing out factors that cause variation or deviation from the normal nine months. It is for this reason that a statement as to reproductive efficiency is of little value without a detailed account of the method used in obtaining it.

No attempt has been made to forecast future events. There has been no effort to prove a preconceived theory. The work consists of assembling related facts in such a way that a large amount of data can be grasped and understood.

This paper calls attention to failures that demand investigation and correction. This phase of the work is not intended to show how failure can be corrected; it simply points out the extent to which failures exist.

The methods described enable one to compute the data at hand and to explain the facts to others interested in problems of a similar nature.

OTHER METHODS OF DETERMINING REPRODUCTIVE EFFICIENCY

There are other methods commonly used to determine the approximate reproductive efficiency of a herd.

Reproductive Efficiency Based upon Number of Services.—Any effort to arrive at a

TABLE III

BULL "A"

No. Services – by Years – Successful and Unsuccessful

Method	1923 S	1923 U	1924 S	1924 U	1925 S	1925 U	1926 S	1926 U	1927 S	1927 U	1928 S	1928 U	1929 S	1929 U	Total
Hand Breeding	–	–	1	1	8	2	7	0	6	1	4	5	3	3	41
Pasture Breeding	7	1	6	0	5	0	4	0	4	1	4	1	5	0	38
Artificial Insem.	–	–	–	–	–	–	–	–	–	–	–	–	1	1	2
Total	7	1	7	1	13	2	1	0	10	2	8	6	9	4	81

TABLE IV

Reproductive Efficiency in Percent

Method	1923	1924	1925	1926	1927	1928	1929	Total	Aver	Wt.	Wt. × Av.
Hand Breeding	--	50.	80.	100.	85.9	44.4	50.	410.3	68.4	41	2804.4
Pasture Breeding	87.5	100.	100.	100.	80.	80.	100.	647.5	92.5	38	3515.
Artificial Insem.	--	--	--	--	--	--	50.	50.	50.	2	100.
Total										81	6419.4
Average											79.2

GRAPH IV

Services 1923–1929

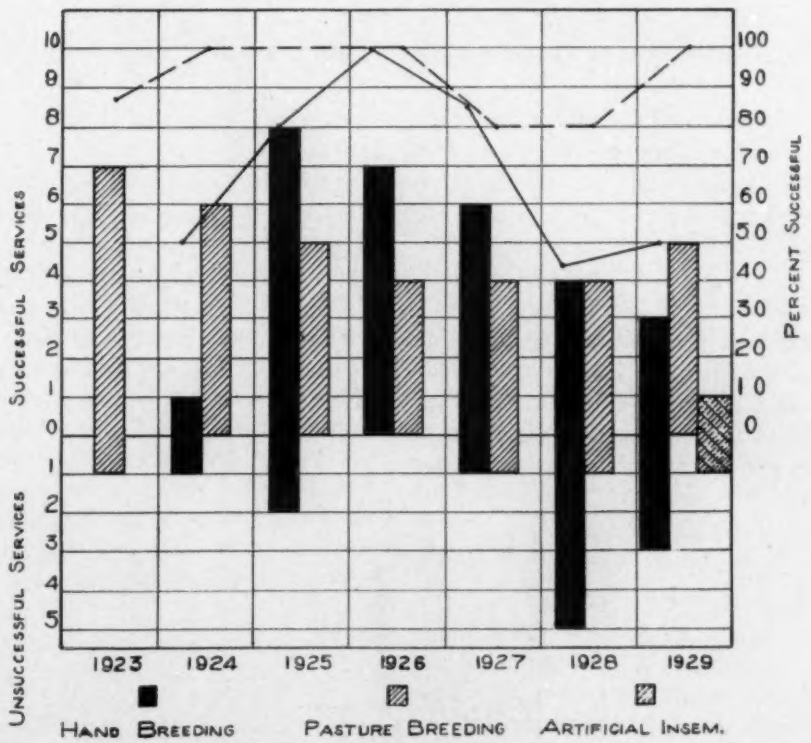


TABLE V

BULL "A"

INDEX OF REPRODUCTIVE EFFICIENCY

METHOD	1923					1924					1925				
	S	PAR	I	WT.	WT. × I	S	PAR	I	WT.	WT. × I	S	PAR	I	WT.	WT. × I
HAND BREEDING						50	60	83.3	25	2082.5	80	60	133.3	67	8931.1
PASTURE BREEDING	87.5	80	109.4	100	10940	100	80	125	75	9375	100	80	125	33	4125
TOTAL				100	10940				100	11457.5				100	13056.1
INDEX					10940					114.58					130.56

METHOD	1926					1927					1928				
	S	PAR	I	WT.	WT. × I	S	PAR	I	WT.	WT. × I	S	PAR	I	WT.	WT. × I
HAND BREEDING	100	60	166.7	64	10668.8	85.9	60	143.2	58	8325.6	44.4	60	74	64	4736
PASTURE BREEDING	100	80	125	36	4500	80	80	100	42	4200	80	80	100	36	3600
TOTAL				100	15168.8				100	12505.6				100	8336
INDEX					151.69					125.06					83.36

S. = PERCENT SUCCESSFUL SERVICES

PAR. = PERCENT SUCCESSFUL SERVICES THAT CAN BE EXPECTED FROM A NORMAL BULL

I. = INDEX = S DIVIDED BY PAR

WT. = WEIGHT = PERCENT OF TOTAL SERVICES

WT. × I. IS COMPUTED TO GET AVERAGE INDEX FOR TWO METHODS

satisfactory solution of the reproductive efficiency through centering the calculation upon number of services can lead only to disappointment. The reasons are obvious. Service may be withheld purposely. The bull may be at fault. In the case of artificial insemination the method and technic may be responsible for failure to conceive. Estrum may take place unobserved, or the opposite condition may obtain; that is, estrum may occur at frequent intervals or be continuous, permitting a service every day.

In the case of pasture breeding the number of services is not a matter of record.

One might continue to relate the inconsistency of the relation of number-of-services-per-calf method of evaluating reproductive efficiency. This does not mean that one should not take cognizance of the number-of-services factor. It brings to light many things of interest and importance.

When conception takes place from one service, both male and female are fertile. If there are repeated failures, one or the other, or both, of the partners are lacking in potency and/or fertility.

The formula for estimating the reproductive efficiency of an individual or herd by this method consists of dividing the number of services by the number of conceptions or the number of calves born, usually the latter, *i.e.*,

$$\frac{C}{S} \times 100 = R_3$$

$$\frac{\text{Calves born}}{\text{Services}} \times 100 = \%\ \text{Reproductive Efficiency}$$

Reproductive Efficiency Based upon the Number of Calves Born During a Twelve-

Month Period.—This method is as follows:

$$\frac{\text{Number of calves born}}{\text{Number of cows in the herd}} = \text{Reproductive Efficiency}$$

$$\frac{C \times 100}{C_r} = R_4$$

This is the common method of determining the reproductive efficiency of a herd, and is the most simple way. It consists of dividing the number of calves born during a twelve-month period by the number of cows in the herd during that period and multiplying the fraction by 100. This gives the result in per cent. Such a method is inaccurate, but it shows the approximate efficiency of the herd. It permits a great variety of discrepancies. One must be careful to describe the method in detail when using the calf-crop basis. It is unfair because it includes cows not in the herd for a nine-month period. It gives no credit to cows which may have been pregnant for as long as eight months, but which calved during the following year. This is made clear by referring to exhibit A, where the months of normal pregnancy and the dates of breeding and calving are shown by diagram.

REPRODUCTIVE EFFICIENCY OF THE BULL

Thus far the analysis has been confined to the cow. Fundamentals established are likewise adaptable to determining the reproductive efficiency of the bull. Since the number of cows in the herd exceeds the number of bulls, the frequency of infertility in the former is more noticeable, but if considered on a percentage basis, the situation may be reversed. It is, therefore, of equal importance to have a yardstick by which the reproductive efficiency of the bull can be determined as well as a recognized system of determining the reproductive efficiency of the cow.

All of the essential data are included in exhibit A. The date of service and the name of the bull are indicated by capital letters. The method of service also is shown. In the case of pasture breeding, a quarter circle appears under the code letter of the bull. Artificial insemination is differentiated by a triangle in the lower right hand corner. Unless otherwise designated, all other services are by hand breeding. The data relating to bulls are taken from exhibit A and arranged in an orderly manner for further analysis, as indicated in table III. It shows the total number of successful and unsuccessful services by each method of breeding. The breeding efficiency of the bull is obtained by dividing the number of successful services by the total number of services and multiplying by 100. The result is expressed in per cent. (See table IV.)

Graph IV is a combination of tables III and IV.

In pasture breeding the number of cows with the bull must be taken as the numerator of the fraction, since there is no method of determining the number of pasture breedings. The foregoing factor must be given due consideration when comparing the reproductive efficiency of a bull by different methods of breeding, or when comparing the reproductive efficiency of different animals by the same method of breeding. In this connection, an arbitrary value was selected for a basis of comparison designated as par. For pasture breeding the value 80 was selected and for hand breeding, the value 60. The index for each method is the degree above or below par and is computed by dividing the reproductive efficiency based on the percentage of successful services by the value designated as par. This can be shown as $\frac{S}{P} \times 100$.

The index for more than one method with the same animal during the same period of time is the weighted average of the indices. The number of services per 100 services is used for the weight. That is, if 75 services were by hand breeding and 25 services were by pasture breeding, these values are the respective weights. The weights are used to give each method an equal position in the calculation.

In table V artificial insemination is excluded because its success or failure de-

pends upon too many factors which are not directly connected with the bull's potency. There should be no objection, however, to using this method in comparing two animals both of which were used exclusively for artificial insemination.

COMMENT

Progress toward the solution of the infertility problem in cattle has been slow. Advancement depends upon our ability to make use of fundamental facts about reproduction.

No other phase of veterinary practice demands so great an effort to master the fundamental factors upon which the solution of the problem is based. The value of accurate and complete records in this field of endeavor can not be overemphasized. Furthermore, records become voluminous and without a great deal of value unless they are arranged in an orderly manner, condensed to a degree that makes them understandable and analyzed by standard methods.

Studies on Equine Encephalomyelitis. II. A Nonantigenic Strain of Pigeon-Brain Virus*

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A RELATIVELY HIGH concentration of virus in the tissue is regarded by many investigators as an essential in the preparation of an antigenic encephalomyelitis vaccine. According to Zinsser,[1] successful active immunization with certain killed virus agents may be largely a function of virus concentration in such diseases as variola, herpes, psittacosis and rabies, as well as rickettsial infections. However, the factor of virus concentration *per se* may not be the only essential index of the antigenic properties of an inactivated encephalomyelitis tissue virus. Evidence in support of this view came to our attention in studies on two strains of western equine encephalomyelitis virus in pigeons. Both strains were consistently passaged by intracranial inoculation of 0.1 cc. of a dilution to 1:1,000,000 of brain-tissue virus.

Following ten successive pigeon transfers of equine encephalomyelitis virus strain 11399, nine pigeons were inoculated intracranially with 0.1 cc. of dilutions ranging from 1:1,000,000 to 1:1,000,000,000 of pigeon-brain virus. The minimum lethal dose of virus approximated 0.1 cc. of a dilution of 1:1,000,000,000 (table I). This virus strain proved lethal to guinea pigs upon intracranial inoculation of 0.1 cc. of dilutions ranging from 1:1,000,000 to 1:100,000,000.

A 20 per cent suspension of the pigeon-brain-tissue virus was inactivated at room temperature for two days by the addition of 0.5 per cent formalin. Following safety and purity tests which showed the virus to be inactive and free from bacterial contamination, an effort was made to measure its antigenic properties by injecting twelve guinea pigs subcutaneously at weekly intervals with 0.5 cc. of the vaccine. Two weeks following the second dose the vaccinated guinea pigs along with four unvaccinated controls were inoculated intracranially with 0.1 cc. of homologous pigeon-brain virus in a dilution of 1:500. All vaccinated and all control guinea pigs died on or before the fifth day (table II).

Another potency test on this vaccine was run with twelve more guinea pigs, and

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†Assigned by the Illinois State Department of Agriculture to the Division of Animal Pathology and Hygiene to assist in diagnosis and research.

[1]Zinsser, H.: The immunology of infections by filtrable virus agents. Virus and rickettsial diseases (Harvard Univ. Press, Cambridge, Mass.), pp. 89-117.

TABLE I—Titration of Strain 11399 Western Equine Encephalomyelitis Virus from Pigeon Brain (Lot 12020—Composite of 25 Pigeon Brains—Eleventh Transfer)

Pigeon No.	Dilution*	Result — Days After Inoculation						
		1	2	3	4	5	6	7
1	1 — 1,000,000	H	H	S	D	..	..	..
2	1 — 10,000,000	H	H	S	D	..	..	..
3	1 — 50,000,000	H	H	H	S	D	..	..
4	1 — 100,000,000	H	H	H	H	D	..	..
5	1 — 200,000,000	H	D	..	..	..	..	..
6	1 — 500,000,000	H	H	H	H	H	H	H
7	1 — 1,000,000,000	H	H	H	H	D	..	..
8	1 — 1,000,000,000	H	H	H	H	H	H	H
9	1 — 1,000,000,000	H	H	H	H	H	H	H

*0.1 cc. inoculated intracerebrally into each pigeon.
H = healthy; S = sick; D = dead.

TABLE II—Test of Immunizing Properties of Strain 11399 Western Equine Encephalomyelitis Pigeon-Brain Vaccine

Guinea Pig No.	Vaccine	Dosage		Virus Exposure	Result — Days After Exposure									
		1st	2nd		1	2	3	4	5	6	7	8	9	10
1	20% formalized pigeon-brain vaccine (lot 12020—composite of 25 pigeon brains—eleventh transfer)	0.5 cc. subcutaneously to each guinea pig	7 days later 0.5 cc. subcutaneously to each guinea pig	Intracranial inoculation 0.1 cc. pigeon-brain virus (strain 11399) dilution 1:500 14 days after second dose of of vaccine	H	H	S	S	D	..	..	..	..	..
2					H	H	S	S	D	..	..	..	..	..
3					H	H	S	S	D	..	..	..	..	..
4					H	H	S	S	D	..	..	..	..	..
5					H	H	S	S	D	..	..	..	..	..
6					H	H	S	S	D	..	..	..	..	..
7					H	H	S	S	D	..	..	..	..	..
8					H	H	S	S	D	..	..	..	..	..
9					H	H	S	S	..	..	..	..	..	..
10					H	H	S	S	D	..	..	..	..	..
11					H	H	S	S	D	..	..	..	..	..
12					H	H	S	S	D	..	..	..	..	..
13	Controls; no vaccine				H	H	S	S	D	..	..	..	..	..
14					H	H	S	S	D	..	..	..	..	..
15					H	H	S	S	D	..	..	..	..	..
16					H	H	S	S	D	..	..	..	..	..

H = healthy; S = sick; D = dead.

TABLE III—Titration of Strain 11399 Western Equine Encephalomyelitis Virus from Pigeon Brain (Lot 12451 Composite of 20 Pigeon Brains—Eleventh Transfer)

Guinea Pig No.	Dilution*	Result — Days After Inoculation											
		1	2	3	4	5	6	7	8	9	10	11	12
1	1:1,000,000	H	H	H	H	H	S	D	..	..	..	..	..
2	1:10,000,000	H	H	H	H	D	..	..	..	..	..	..	..
3	1:50,000,000	H	H	H	H	H	H	S	D	..	..	..	..
4	1:100,000,000	H	H	H	H	H	H	S	S	D	..	..	..
5	1:200,000,000	H	H	H	H	D	..	..	..	..	..	..	..
6	1:500,000,000	H	H	H	H	D	..	..	..	..	..	..	..
7	1:1,000,000,000	H	H	H	H	H	H	S	S	S	S	S	D
8	1:1,000,000,000	H	H	H	H	D	..	..	..	..	..	..	..
9	1:1,000,000,000	H	H	H	H	H	H	S	D	..	..	..	..

*0.1 cc. inoculated intracerebrally into each guinea pig.
H = healthy; S = sick; D = dead.

again all vaccinated and control guinea pigs died following the challenging exposure.

The nonprotective action of this lot of vaccine was unexpected, since vaccine made from another western strain of equine encephalomyelitis virus (6289) was measurably protective (Graham and Levine[2]) and since the concentration of virus as determined by pigeon and guinea pig titrations was higher in strain 11399 than in strain 6289. In order to check the accuracy of the above observations a second lot of vaccine was prepared from the brains of pigeons showing symptoms following intracranial inoculation with virus strain 11399. Titrations revealed that 0.1 cc. of a 1:1,000,000 dilution of the pooled pigeon-brain virus inoculated intracranially was lethal to guinea pigs (table III), while the same virus dilution inoculated intracranially was lethal to pigeons. The end point or minimum lethal dose of vaccine was not established, since higher dilutions were not employed.

The second lot of tissue virus was inactivated at room temperature for two days by the addition of 0.5 per cent formalin. Safety and purity tests proved that the virus was completely inactivated and free from bacterial contamination. Potency tests on guinea pigs as employed in the first lot of vaccine (strain 11399) confirmed the nonantigenic properties of this strain of virus. All vaccinated and control guinea pigs died after administration of the challenging dose of virus.

[2]Graham, R., and Levine, N. D.: Studies on equine encephalomyelitis. I. Antigenic properties of pigeon-brain-tissue vaccine. Am. J. Vet. Res., ii (Oct. 1941), pp. 430-435.

SUMMARY

A strain of western equine encephalomyelitis virus (11399) was passaged through ten successive pigeon transfers by inoculating 0.1 cc. of a dilution of 1:1,000,000 of pigeon-brain-tissue virus. Titration of two separate lots of the virus in the pooled pigeon-brain tissue revealed concentrations of 1,000,000,000 to 10,000,000,000 or more guinea pig-lethal doses and 10,000,000,000 or more pigeon-lethal doses per gram of brain tissue upon intracranial inoculation. Two separate lots of pigeon-brain vaccine prepared from the eleventh pigeon transfer were inactivated by the addition of 0.5 per cent formalin. Neither lot of vaccine was measurably protective in guinea pigs against intracranial inoculation of 0.1 cc. of a dilution of 1:500 of homologous pigeon-brain virus.

Equine Periodic Ophthalmia*

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PERIODIC OPHTHALMIA IS an inflammatory disease of the eyes of Equidae characterized by recurring, acute symptoms separated by quiescent periods of varying duration. The disease is primarily an iridocyclitis. After repeated attacks the lens, retina and vitreous humor are affected and the vision is impaired. Unqestionably, it is the most common cause of blindness in horses and mules.

This disease, which is generally called periodic ophthalmia, might better be named recurrent iridocyclitis to be more precise. There are many other terms used as synonyms. Among them are recurrent uveitis, anterior uveitis, recurrent ophthalmia, specific ophthalmia, moonblindness, mondblindheit (German), fluxion periodique (French), monatblindheit (German) and iridochoroiditis recidiva. Greenfield[1] lists several others: panophthalmitis infectosa (toxica) recidiva equi, recurrent iritis, iridochoroiditis, iridochoroiditis recurrens, remittant ophthalmia, rheumatoid ophthalmia, constitutional ophthalmia, ophthalmitis, and lunatic disease. Recurrent iridocyclitis is preferable because it more clearly designates the structure primarily involved, but since the term periodic ophthalmia is the more common one, we shall use it in this paper. When the knowledge of its etiology becomes more complete, the terminology may have to be revised.

Periodic ophthalmia was one of the early diseases of animals to be recognized and described. Vegetius described it in the fourth century, A.D. It was then thought to be caused by the changes of the moon. Writers of the eighteenth, nineteenth and twentieth centuries also focused attention on periodic ophthalmia.[2] At the present time it is again arousing much interest. Periodic ophthalmia is important because of the toll it takes among horses and mules. It is also important in the study of similar conditions of obscure cause in the human eye.

Published information on the various aspects of periodic ophthalmia is scattered through many periodicals. It is our purpose in this paper to assemble the best of this material, to examine it critically and to present some hitherto unpublished observations of our own.

EPIZOÖTIOLOGY

Periodic ophthalmia probably occurs throughout most of the world. It is known to occur in the United States, South Africa, Italy, Argentina, Germany, Panama, Hawaii and the Philippine Islands. Although previously believed to be a disease not found in Australia, the recent report of Rose[3] indicates that the disease does occur in that country.

Estimates as to its incidence vary a great deal. Statistics[4] of the United States Army between the years 1923 and 1935, inclusive, show that the average incidence in military animals in the United States during that 13-year period was 6.11 per thousand horses and 2.03 per thousand mules. For the same period among animals in the Philippines, the incidence was 23.79 per thousand horses and 8.23 per thousand mules. The highest incidence in military animals was found in those stationed in the upper Mississippi River Valley and the

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[1]Greenfield, H.: Irido-Cyclitis (ophthalmitis) amongst animals at war. Vet. J., xcvi (May 1940), pp. 178-189; xcvi (June 1940), pp. 232-242.

[2]Merillat, L. A.: Periodic ophthalmia. No. Am. Vet., xi (Aug. 1930), pp. 27-32.

[3]Rose, A. L.: Does periodic ophthalmia of horses occur in Australia? Australian Vet. J., xvii (1941), pp. 19-20.

[4]Caldwell, G. L.: Statistics. U. S. Army Vet. Bul., xxxi (Jan. 1937), pp. 14-18.

Ohio River Valley. There was a much lower incidence in the Rocky Mountains, Inter-Mountain, West Coast and Southwest regions of the United States. The disease was of enough importance to the allied armies of World War I to cause them to discuss it along with three other diseases at the British-French Conference of Veterinary Officers on January 12, 1918.[5]

It is usual for the disease to spread slowly. Ordinarily, just a few animals of a group become affected at any one time, although it does not tend to spread through a band of horses or mules. If one case is left in a group, other cases are apt to appear from time to time. On the other hand, in some groups, including both infected and noninfected animals, many clean animals may remain exposed for long periods without contracting it.

Less frequently, but by no means rarely, there may be sudden outbreaks involving a high percentage of the animals in a group. Progressively increasing numbers of cases frequently occur in a band of horses. Another band, maintained nearby and fed and cared for under identical conditions, but not in contact with the first band, may remain free of the disease. This characteristic seems to indicate the infectious, or at least transmissible, nature of the disease. On the basis of our present knowledge, it appears to be a good practice to isolate affected animals, just as if the disease were of known infectious nature.

More cases are observed in horses and mules that have reached maturity (5 to 6 years old). However, all ages are susceptible. We have observed two typical cases in animals under 1 year of age. The first attacks occurred when the foals were 3½ and 7 months old, respectively. In each case the symptoms, course and lesions were typical of periodic ophthalmia. Old cases, exhibiting chronic degenerative lesions, are most frequently seen in horses past 6 years of age. However, old cases are not entirely limited to the older age group.

[5]Newsome, A. C.: Periodic ophthalmia. Vet. J., lxxiv (1918), pp. 157-161.

According to Jakob,[6] a similar disease occurs in cattle in Germany. American literature contains no supported evidence that the disease occurs in cattle in the United States. The prevailing opinion is that it is limited to Equidae.

Greenfield[1] states that a clinically identical disease occurred in camels in Waziristan during World War I. He observed 1,000 cases in camels at that time. This number represented 10 per cent of the total camel strength in the British military forces. He further states that at least 6,000 cases occurred in the animals of the British Expeditionary Force during World War I and that as much as 10 per cent of some divisions were affected.

Smith[7] records some interesting observations made during the South African War of 1889 to 1902. During those years, 109,739 horses were exported from the United States to South Africa. According to Drs. Hutcheson and Theiler, this disease was unknown in South Africa prior to the importation of horses from the United States. In 1901, two years after the arrival of the first animals from the United States, a marked increase in the incidence of periodic ophthalmia was observed. One remount depot was 48 per cent affected. The movement of ophthalmia cases from one remount depot to two others resulted in an outbreak at the latter depots. In the second year after the war, 14 per cent of all of the military animals in South Africa were affected. This represented over 2,000 cases. After the fourth year following the war, the disease disappeared from South Africa.

EXAMINATION OF THE EYE OF THE HORSE

Since thorough examination of the ocular structures is important in the diagnosis of periodic ophthalmia, a discussion of the methods of examination that are of value in veterinary medicine is included in this

[6]Jakob, H.: Pathologishe Anatomie des Auges der Tiere. Lubarsch-Ostertag Ergebnisse der Allg. Path. u. Path. Anat., Erganzungsband I, xxi (1927), pp. 77-79, 81-83.

[7]Smith, E.: Veterinary history of the war in South Africa, 1889-1902 (H. and W. Brown, London, 1919), pp. 287-288.

paper. Several authors, especially Gibbons[8] and Love,[9] recently described methods of examination and normal phenomena in the equine eye. We have consulted the work of these authors as well as standard texts on ophthalmology in preparing this section. We also draw from our own and our colleagues' experiences in inspecting horses for soundness prior to purchase by the Army.

As is true in all diagnostic procedures, systematic methods are essential in examining the eyes and their adnexa. In general, methods of ocular examination are divided into two groups: (1) examination with the unaided eye and (2) examination with the use of some visual aid. Among the methods used in the second group are direct ophthalmoscopy, indirect ophthalmoscopy, transillumination, focal illumination, observation of Purkinge-Sanson's images and slit-lamp microscopy.

Unaided Eye.—Suitable light is important. Uniform daylight, but not direct sunlight, is desirable. By backing the animal into a doorway on the shady side of a building, good light is usually secured and much valuable information obtained. Here one notes the eyelids, orbit and anterior parts of the eyeball itself. The eye should be full, the cornea glistening, the lids smooth and free from skin blemishes and excessive wrinkling. The head should be held in an alert posture and there should be no unusual twisting or turning in the effort to see. Tears will not normally be seen on the cheek, nor should pus or foreign matter be present in the lacrimal sac at the medial canthus.

Absence of excess lacrimation or exudates at the medial canthus usually means the nasolacrimal duct is open and functioning properly. Wounds or abrasions of the lids, fractures, and deformities of the orbit should be looked for. By pressing the index finger on the upper lid between the bulb and orbit and pressing simultaneously with the thumb of the same hand on the lower lid, the conjunctiva can be partially exposed and the third eyelid may be caused to protrude. Excess vascularization of the scleral conjunctiva, congestion or localized hyperemia, foreign bodies and injuries should be looked for at this stage.

An estimate of the intraocular tension should be arrived at by digital palpation of the bulb through the upper eyelid. By placing the finger over each eye simultaneously, a practical comparison of the tension in each bulb may be made.

The eye itself may now be studied. The cornea should be smoothly convex and free from opacities and visible blood vessels. If

Fig. 1. Method of direct ophthalmoscopy.

corneal lesions are suspected, fluorescein (2%) or mercurochrome (2%) will stain any corneal lesions and make them clearly visible. The iris is of a uniform soft, velvety brown color in most normal horses. In albino or partial albino horses, however, unilateral or bilateral absence of iris pigment may be observed (so-called watch eye). The iris normally has a large mass of black pigment (granula iridis, or corpora nigra) on its dorsal pupillary margin and a similar but much smaller mass on the ventral pupillary margin. The equine pupil in bright light is slit shaped, with the length of the slit horizontal. As the pupil dilates it becomes oval. When fully dilated it is evenly circular. The pupil should contract rather quickly when exposed to bright

[8]Gibbons, W. J.: The physical examination of the eye of the horse. Cornell Vet., xxvii (1937), p. 348.
[9]Love, W. G.: The use of the ophthalmoscope in veterinary practice. Univ. of Pennsylvania Vet. Ext. Quart., xl (Jan. 16, 1940).

light, and dilate as promptly when light is excluded. In the equine eye the pupillary response is prompt, but not instantaneous. Of prime importance is the equal response of the pupil of each eye to an equal amount of light. Failure of the pupil to dilate is a common indication of posterior synechia. Unequal dilatation of different parts of the pupil indicates the same condition. The iris should not be torn, nor should bits of iris pigment adhere to the anterior lens capsule. If large or dense opacities are present in the lens, they usually can be seen by this naked-eye examination.

Direct Ophthalmoscopy.—The accessory and ocular structures as far back as the iris can be examined fairly satisfactorily with

Fig. 2. A type of ophthalmoscope.

the unaided eye. To study the structures posterior to this point (lens, vitreous and retina) an ophthalmoscope is essential. The modern instrument (fig. 2) which has been designed for use by the ophthalmologist is satisfactory for our use. In fact, the use of some type of ophthalmoscope is necessary for accurate and complete examination. As an aid to the diagnosis of diseases other than those of the eye (especially anemias, acute septicemias, arterial disease) an ophthalmoscopic examination of the fundus is often of real value.

The ophthalmoscopic examination is conducted in as near complete darkness as possible. The exclusion of all external light is ideal, but usually a satisfactory examination can be made if the light is reduced to the extent found possible in a closed stable. Prior dilatation of the pupil is a distinct aid, especially to the beginner. One per cent atropine solution instilled on the conjunctiva an hour or so before the examination suffices in normal eyes.

The usual ophthalmoscope consists of a light source combined with a series of lenses of varying strengths which can be easily moved into position for observation through them. Each lens is numbered and has a plus or minus symbol. The plus lenses are used for observation of the anterior part of the eye; the minus lenses for the deeper structures. If the observer has a normal (emmetropic) eye, theoretically no lens should be needed to see the retina. In studying an equine eye, however, and also due to the involuntary accommodative spasm of most observers' eyes, a —3 lens usually is required to see the optic disc clearly. If the optic disc can not be seen sharply, it means either that some opacity, or an error in refraction of the ocular media, prohibits reflected light from the disc from reaching the observers' eye. If changing the lenses does not bring the retina into sharp focus, some opacity of the media may be assumed to exist. The ophthalmoscope must be held very close to the observer's eye, and within a few inches of the eye of the patient (fig. 1).

The normal equine fundus oculi (fig. 4) presents several distinct characteristics. The retina has a dorsal, iridescent zone called the tapetum lucidum which is separated by a horizontal line from a ventral, dark brown area, the tapetum nigrum. A short distance below the ventral border of the tapetum lucidum, the head of the optic nerve may be seen as it enters the eyeball. This is the optic papillus or disc. The equine disc is nearly circular, and usually of orange color. Blood vessels radiate from the periphery. The arteries can not be distinguished from the veins in the equine eye. Occasionally a blood vessel may be visible in the disc. Frequently a ring of white partially surrounds the disc. This is the edge of the sclera showing through a depigmented zone in the chorioid. The tapetum lucidum usually is made up of yellowish and greenish areas surrounding numerous small black puncta. Toward the dorsal part, mixtures of brown color appear. Frequently there are variations in the appearance of the normal fundus, and experience is highly important in determining the normal from the abnormal.

The vitreous body, lens and aqueous humor are observed by changing to one of

the plus lenses of the ophthalmoscope. Usually the +8 lens is best for observing the vitreous and posterior lens capsule. If the various parts of the media are free from opacities, nothing will be seen. Opacities in the vitreous or lens, if present, appear as black masses of varying sizes and shapes due to the fact that they interrupt the reflections of light from the retina back to the observer's eye.

Location of Opacities.—Of particular diagnostic importance is the location of opacities that may be present in the media. With experience an accurate estimate of the location of opacities may be arrived at with the ophthalmoscope. This is diagrammatically represented by figure 3. The location of an internal ocular lesion is determined by the apparent movement of the lesion resulting from changes in position of the observer's eye in relation to the eye of the patient. In examining the eyes of animals this is accomplished by movements of the observer while the patient's eye remains in a fixed position. In figure 3, observation from point A toward point B may reveal opacities at 1, 2, 3 or 4. If the observer moves from point A to point C (broken arrow), opacities 1 and 2 in front of the center of the lens will appear to move in the opposite direction (dotted arrows). At the same time, opacities in the rear of the lens (3 and 4, heavy arrows) will appear to move in the same direction as the observer. The latter is due to the image-reversal effect of the lens. Relative positions in the various parts of the vitreous are estimated from the amount of apparent movement each opacity appears to undergo.

Lesions in the Fundus.—Lesions in the fundus may be circumscribed or diffuse, single or multiple. For purposes of description, diagnosis and record, a common method is used to describe the lesions. Most lesions, such as hemorrhagic, congested or depigmented areas, are described by noting their relation in size and location to that of the optic disc. The familiar locations of time indications of the clock are usually employed. Hence, lesions are described as "at 12 o'clock," "3 o'clock" and so forth. The horizontal line separating the tapetum lucidum from the nigrum is also of value as a landmark in the equine eye. Circumscribed lesions in the fundus are measured by comparison to the size of the disc. The abbreviation commonly used is p. d., for papillary diameter. Thus, an area of choroiditis, for instance, may be described as 2 p. d. in size and 3 p. d. from the disc toward 9 o'clock. It may be further located by stating that it is ½ p. d. below the ventral border of the tapetum lucidum.

Refraction.—Refraction of light is the deflection of a ray which occurs when it passes from one medium into another of different density. This deflection depends upon the density of the medium (refractive index) and the nature of the surfaces

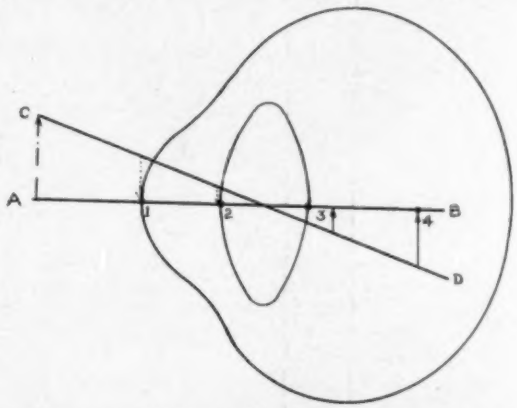

Fig. 3. Location of opacities in ocular media.

of that medium (concave, convex, etc.). A ray of light striking a medium perpendicular to its surface is not refracted. The refractive media of the eye (cornea, aqueous, lens, vitreous) have the function of bending, or refracting, the light rays that reach them in such a way as to produce an image on the retina. If these media do not bend the light rays in such a way as to produce a sharp image at the retina, a refractive error is present.

The measurement of the amount of refractive error and its correction with prosthetic lenses (glasses) is an important part of ophthalmology as far as the human eye is concerned. In animals, however, it is not as important to determine the amount of refractive error, since glasses are not used. A marked refractive error usually

can be detected with the ophthalmoscope and, of course, would affect the vision. Most lesions in equine eyes are due to opacities which prevent the passage of light to the retina, and not to refractive errors caused by anatomic factors. We do not consider the correction of refractive errors in equine eyes of sufficient importance to merit description here.

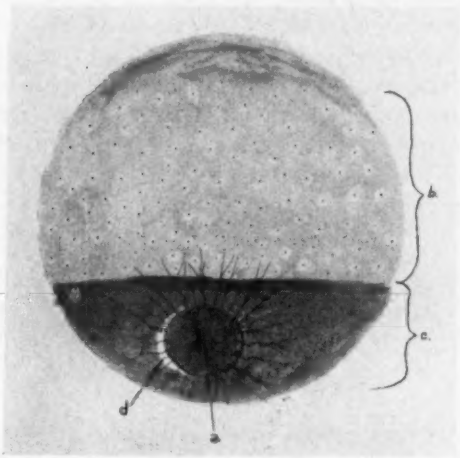

Fig. 4. Normal equine fundus oculi—ophthalmoscopic view, enlarged. (a) Optic disc, (b) tapetum lucidum, (c) tapetum nigrum, (d) scleral edge around disc.

Indirect Ophthalmoscopy.—Indirect ophthalmoscopy is performed by the use of the ophthalmoscope in combination with a convex lens of about 4 to 5 diopters. This convex lens is held 5 to 6 inches in front of the eye to be examined. The ophthalmoscope is held about 30 cm. in front of this lens and its light reflected through the lens into the patient's pupil. This light is reflected out through the pupil and made convergent by the convex lens, forming an inverted image between the lens and the observer's eye. The observer moves forward in order to bring this image into sharp focus. With the +8 lens of the ophthalmoscope, a magnification of about 5 diameters is secured. This method has no advantage over the direct method for most examinations; hence it is used very little in veterinary medicine.

Transillumination.—In locating intrabulbar neoplasms and in other similar conditions, transillumination of the bulb is used occasionally. This is performed by applying a small but bright beam of light to the sclera at various points and observing the color and intensity of the light reflex in the pupil. The Lange lamp is expressly designed as a light source for this purpose. Dense masses of tissue, such as neoplasms, inside the bulb will interfere with the passage of the light. Hence, such lesions may be located and measured by this method.

Focal Illumination.—Focal illumination is the application of a small beam of light to various parts of the eye as an aid to observation. Any small light that can be focused is used. The light from an ordinary small flashlight equipped with a clear bulb is usually quite satisfactory. It should be possible to focus the light beam at 6 to 8 inches from the bulb. Small bits of pigment on the lense capsule and small opacities in the cornea are often recognized much more quickly when this narrow beam of light is directed upon them from various angles. The light is also useful in illuminating the iris. This method of examination should be utilized to supplement the ophthalmoscope whenever possible. Used alone, it also may yield information of value.

Purkinge-Sanson's Images.—These are the images formed by the cornea, anterior lens capsule and posterior lens capsule when a light is held in front of the eye. The corneal image is upright and brilliant; that of the anterior lens capsule is upright, smaller and less intense than that of the cornea. The deepest image is smaller and less intense, and is inverted. The sharpness and intensity of these images give some clue as to the clearness of the refractive media through which the light rays pass. This method is not of particular importance.

Slit-Lamp Microscopy.—Slit-lamp microscopy as now used is generally a combination of corneal microscopy and slit-lamp illumination. The slit lamp produces a narrow beam of focal light that is used to illuminate the various parts of the re-

fractive media. Observation through a microscope with magnification of 16 to 80 diameters reveals the media in optical section. By its use the ophthalmologist is able to locate and study many lesions in the eye long before they can be recognized by any other means. Microscopic study in the living subject of opacities in the cornea, cells in the aqueous, or early changes in the lens or vitreous is possible. This instrument is expensive. Its use on animals has not been reported.

ETIOLOGY

The etiology of periodic ophthalmia has not been established indisputably. Speculations as to the cause have been legion. We shall discuss some of the most prominent theories and try to indicate the present status of each.

Bacterial Organisms.—Almost since the relationship of bacteria to disease first became known, attempts have been made to isolate bacteria from equine eyes affected with periodic ophthalmia. A great variety of microörganisms have been described as being associated with the disease. Law[10] states that Potapenke found a plasmodium in the blood; Vigezzi found a "micrococcus" in the aqueous; Trichera found a bacillus and cocci in the aqueous; and Robert Koch cultured affected aqueous and isolated a bacillus and cocci singly or in chains. In 1919, Dalling[11] described a "nerve bacillus," but Knowles[12] later indicated that this

[10]Law, J.: Textbook of veterinary medicine (Ithaca, N. Y., 1901), iii, pp. 404-420.

[11]Dalling, T.: Investigations on specific ophthalmia. Vet. J., lxxv (1919), pp. 16-24.

[12]Knowles, R. H.: Observations on recurrent ophthalmia. J. Comp. Path. & Ther., xxxii (1919), pp. 192-196.

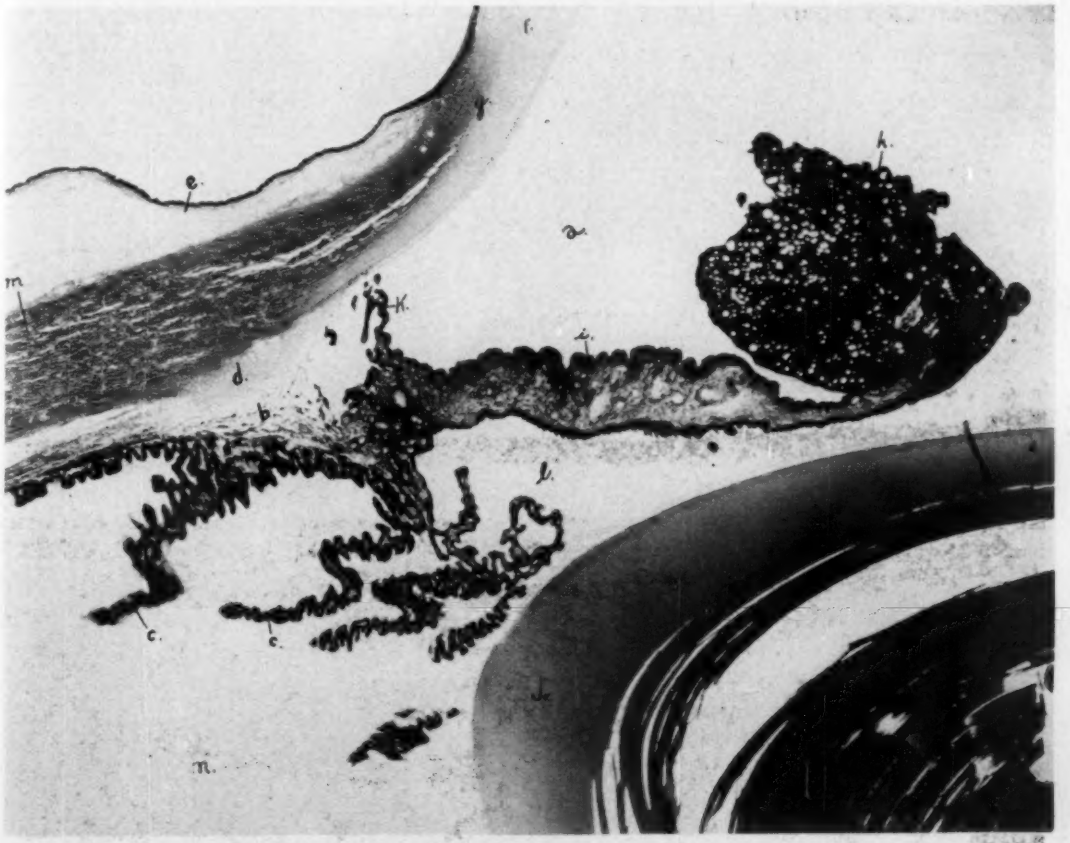

Fig. 5. Normal equine eye—meridional section through dorsal limbus (x 15). (a) Anterior chamber, (b) ciliary body, (c) ciliary processes, (d) ciliary muscle, (e) conjunctiva, (f) cornea, (g) corneo-scleral junction, (h) granula iridis, (i) iris, (j) lens, (k) ligamentum pectinatum, (l) posterior chamber, (m) sclera, (n) vitreous body.

organism has no etiologic significance. In 1928, Rosenow[13] isolated a gram-negative bacillus from the conjunctival sacs of affected horses. He also isolated this organism from noninfected eyes as well as from forage and water troughs. He called it *Flavobacterium ophthalmiae* and suggested that it has etiologic significance. Woods and Burky[14] as well as several others later proved that this organism does not cause periodic ophthalmia.

In 1939, Burky[15] suggested that periodic ophthalmia in horses is a manifestation of brucellosis. He isolated organisms of the genus Brucella from the genital tract of a few mares affected with periodic ophthalmia. He also observed some cases of recurrent iridocyclitis in man that he believed to be due to Brucella infection. Agglutination tests conducted in our laboratory[16] on serums of normal and ophthalmia-affected animals tend to refute Burky's claim. No higher agglutination titer was observed in the diseased horses than in the normal controls. Moreover, many horses affected with periodic ophthalmia did not exhibit agglutinins in dilutions as low as 1:20. Cultural tests were also negative. The work of Stubbs and Love[17] agrees with

[13]Rosenow, E. C.: Bacteriological studies on the etiology of periodic ophthalmia in the horse. J.A.V.M.A., lxxi (1927), pp. 378-383; lxxii (1928), pp. 419-458.

[14]Woods, A. C., and Burky, E. L.: The relationship of the *Flavobacterium ophthalmiae* to periodic ophthalmia of horses. Arch. Ophthalmol., ii (1929), pp. 456-467.

[15]Burky, E. L., Thompson, R. R., and Zepp, Helen M.: The rôle of Brucella in human and animal ocular disease with special reference to periodic ophthalmia in horses. Am. J. Ophthalmol., xxii (Nov. 1939), pp. 1210-1217.

[16]Jones, T. C.: The relation of brucellosis to periodic ophthalmia in Equidae. Am. J. Vet. Res., i (Oct. 1940), pp. 54-57.

[17]Stubbs, E. L., and Love, W. G.: Studies on the relationship of brucellosis to periodic ophthalmia. J.A.V.M.A., xcvii (Nov. 1939), p. 538.

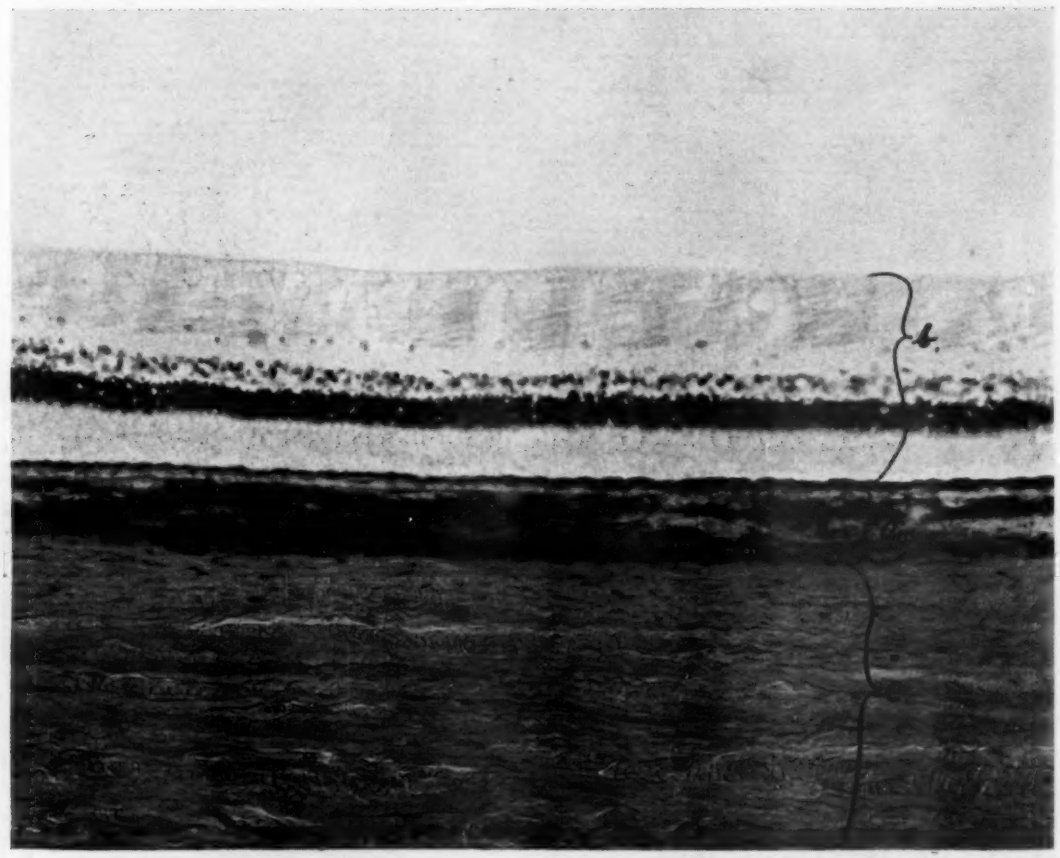

Fig. 6. Normal equine retina, chorioid and sclera (x 165). (a) Chorioid, (b) retina, (c) sclera.

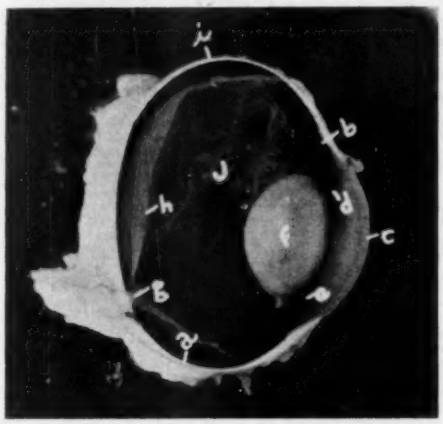

Fig. 7. Normal equine eye—sagittal section cut through optic disc following fixation in formalin. Two artefacts are present: The retina is detached from the chorioid and the vitreous contains numerous air bubbles. (a) Chorioid, (b) ciliary body, (c) cornea, (d) granula iridis, (e) iris, (f) lens, (g) optic disc or papillus, (h) retina, (i) sclera, (j) vitreous body.

our observations. Horses have been shown to develop a high titer to Brucella antigen in known cases of brucellosis.[18-21]

It is obvious that no one visible bacterial organism has been established as the cause of periodic ophthalmia. The organisms that have been encountered were probably either contaminants, secondary invaders, or present as part of a concomitant infection. Further evidence will be necessary before this disease is proved to be caused by any of the bacteria heretofore described.

Ultravirus.—Bossi,[22] in 1928, from clinical and limited experimental observations conducted since 1917, suggested that a filtrable virus may be the cause of periodic ophthalmia. In 1930, Woods and Chesney[23] reported the transmission of this disease by means of a filtrable agent. Using Berkefeld filtrates of macerated equine ocular tissue as their inoculum, they produced a retinitis in rabbits by intravitreous inoculation. After serial passage through six transfer-generations of rabbits, they inoculated rabbit ocular filtrate into a horse's eye. Typical symptoms, with recurrences, resulted. Stubbs and Ratcliffe[24] and later

[18]Carpenter, C. M., and Boak, Ruth A.: The significance of the horse in brucellosis. J. Bact., xxxiii (1937), p. 40.

[19]Deem, A. W.: *Brucella abortus* in horses. J. Bact., xxxiii (1937), p. 40.

[20]Huddleson, I. F.: Brucellosis in man and animals (The Commonwealth Fund, 1939).

[21]Stone, W. S.: Brucellosis in horses. Cornell Vet., xxviii (1938), p. 91.

[22]Bossi, V.: Periodic ophthalmia in horses. (Title trans.) Clin. Vet., li (June 1928), pp. 337-350.

[23]Woods, A. C., and Chesney, A. M.: The transmission of periodic ophthalmia of horses by means of a filterable agent. J. Exp. Med., lii (1930), pp. 637-648.

[24]Stubbs, E. L., and Ratcliff, H.: Studies of recurrent ophthalmia of horses. Univ. of Pennsylvania Bul., Vet. Ext. Quart., xxxviii (Jan. 15, 1938), pp. 3-12.

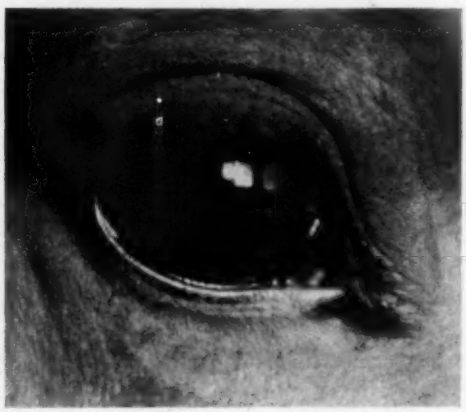

Fig. 8. Normal equine eye. Compare with figure 9. Note that the pupil in moderate light is ovoid with its long axis horizontal. The two bright squares in the dorsal part of the cornea are reflections of the light used in making the photograph and should be disregarded.

Fig. 9. Acute periodic ophthalmia—second day. Note the exudate in the ventral half of the anterior chamber. The pupil and iris are not visible. Marked ptosis and photophobia made it necessary to open the lids with the finger. The bright spot in the dorsal part of the cornea is the reflex of the light used in photographing the eye and should be disregarded.

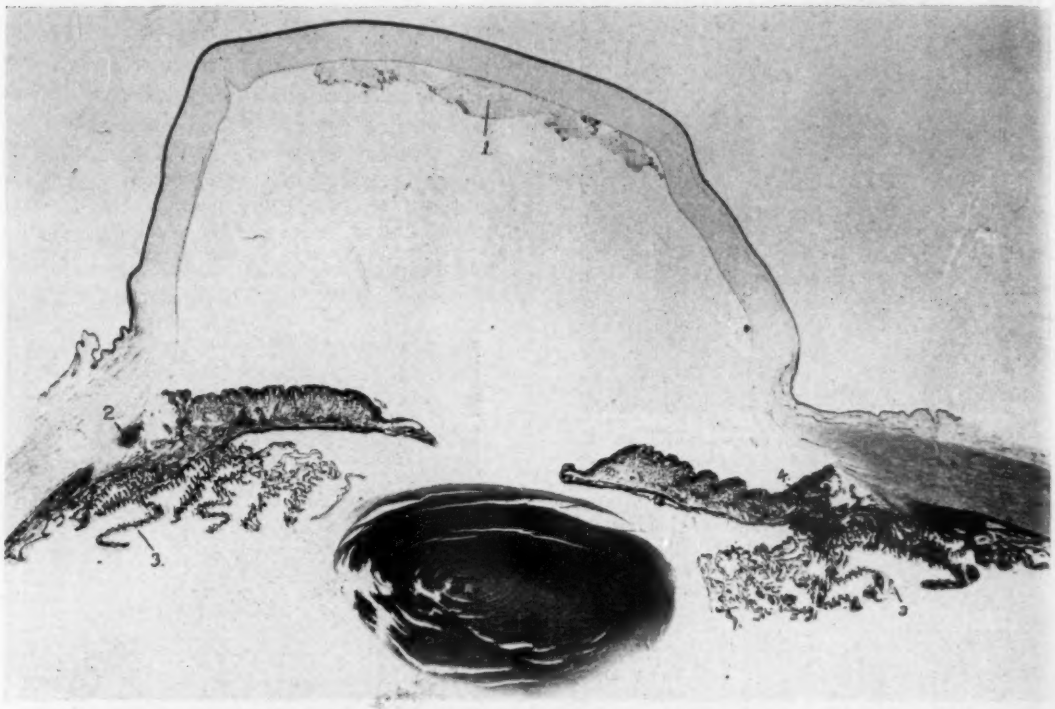

Fig. 10. Acute periodic ophthalmia—meridional section (x 6). Some shrinkage of the globe (note in cornea) occurred in preparation and is not a part of the pathologic lesions. Note the exudate in the anterior chamber (1), in the ciliary body (2), ciliary processes (3) and iris (4).

Stubbs and Murphy[25] partially confirmed these observations, but in their experiments a disconcerting number of control animals developed symptoms similar to those of periodic ophthalmia. These experiments are probably the best that have been conducted relative to the etiology of this disease, but their results are not conclusive. Sensitization of the uvea to the products injected has not been eliminated, and many control animals inoculated with materials from normal eyes developed confusing symptoms and lesions.

The symptoms and lesions of this disease strongly suggest that an infectious agent is its cause. Epidemiologic observations and the absence of constant bacteria further strengthen the filtrable virus theory. However, until we can regularly transmit this disease and can study the virus in experimental animals or tissue cultures, we can not claim that periodic ophthalmia is caused by an ultravirus.

Heredity.—Some authors believe that this disease is hereditary. Others[26] have indicated that a hereditary susceptibility may be of importance along with some unknown factor. It has been observed that many stallions and mares with periodic ophthalmia have been mated and normal foals (which grew into normal adults) have resulted. Except in isolated instances it has been difficult to establish any definite evidence of hereditary transmission. The symptoms and lesions also weaken the hereditary theory. As a rule, acute inflammatory diseases are not hereditary. However, the causative agent could possibly be transmitted from one generation to the next by contact. Further studies relative to possible genetic factors in this disease are needed. Such studies are now being conducted by the U. S. Army Veterinary

[25]Stubbs, E. L., and Murphy, J. M.: Studies of recurrent ophthalmia of horses. Univ. of Pennsylvania Bul., Vet. Ext. Quart., xxxviii (June 15, 1938), pp. 5-18.

[26]Schäper, W.: Entstehung und Bekämpfung der Mondblindheit im Lichte der Konstitutionsforschung. Tierarztl. Rdsch., xliii (1937), pp. 283-288.

Research Laboratory and the Surgeon General's Office.

Nutrition.—It often has been theorized that feeds and feeding methods might have something to do with this disease.[27] The fact that vitamin A deficiency is known to produce ophthalmic symptoms in rats has led to suggestions that such a deficiency might be present in periodic ophthalmia. On the other hand, the pathologic lesions seen in xerophthalmia and riboflavin deficiency are different from those seen in periodic ophthalmia. Further, it is a common observation that near ideal dietary conditions do not prevent severe outbreaks of the disease, nor do very poor diets seem to bring on attacks. Until some experimental evidence is developed to indicate that the contrary is true, we are forced to conclude that nutrition plays no major rôle in periodic ophthalmia.

In 1939, Tagliavini[28] found what he believed to be a thyroid deficiency in 50 cases of the disease. He concluded that the thyroid deficiency was the cause. Autopsies on 40 typical cases at our laboratory did not reveal any gross or microscopic pathology in the thyroid gland. The recent studies of Seibold[29] substantiate our observations. It is probable that the changes in the thyroid were merely concomitant with those in the

[27]Bonazzi, A., and Merillat, E.: Studies on periodic ophthalmia in the horse. Vet. Med., xvii (1922), pp. 213-218, 256, 267-274, 358-359.

[28]Tagliavini, A.: Ueber die rezidivierende Uveitis des Pferdes. Arch. f. Tierheilk., lxxiii (1939), pp. 66-70.

[29]Seibold, H. R.: Report on the relationship of thyroid activity to periodic ophthalmia. Am. J. Vet. Res., 1 (Oct. 1940), pp. 52-53.

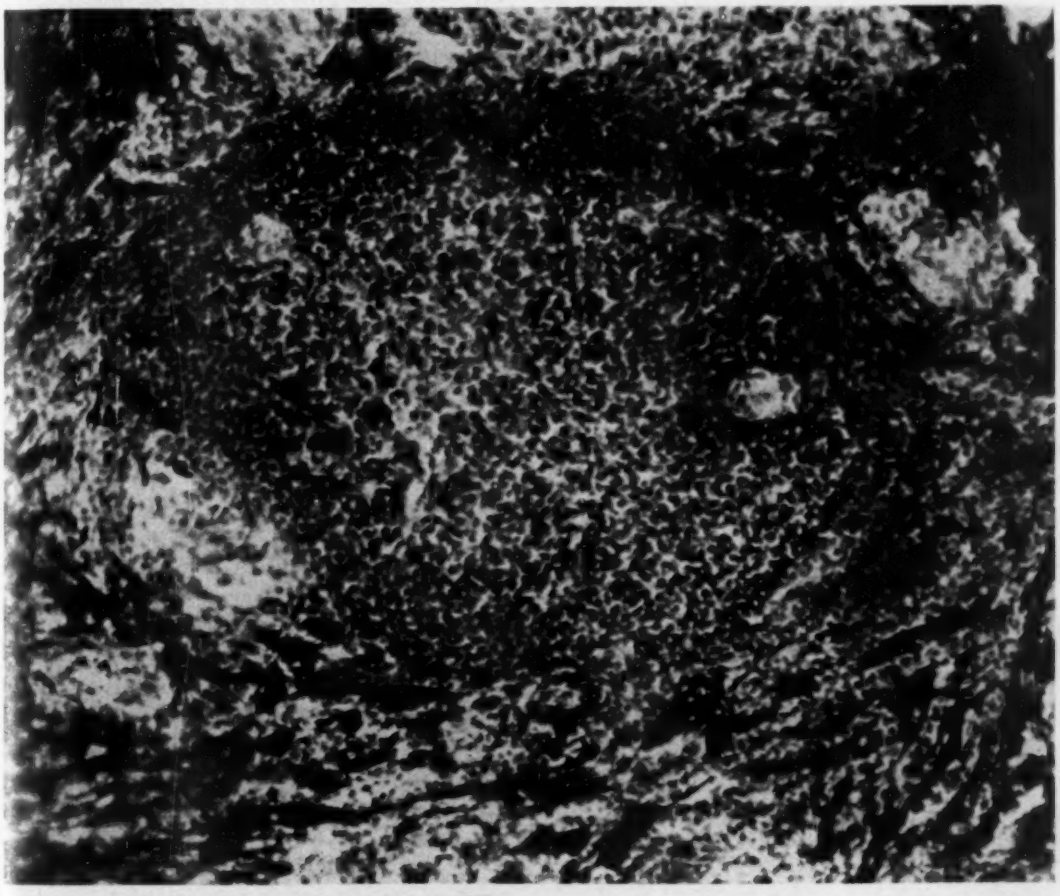

Fig. 11. Acute periodic ophthalmia (x 260)—an enlarged view of (4), figure 10. A collection of lymphoid cells surrounding a capillary in the iris stroma.

eye, and actually not the cause of the latter.

Hypersensitiveness.—Several writers have suggested that periodic ophthalmia may be a reaction due to local tissue sensitization to a toxin of some kind. Gmelin[30] favors the opinion that intestinal parasites (ascarids and strongyles together) may produce a toxin that has an affinity for the uveal tissues. Manninger[31] states that various products of protein decomposition, when inoculated into one eye, may produce lesions in the opposite eye. The early work of Guillery[32] showed that the uvea may be sensitized to many different toxic protein products (uveagifte). Poos[33] adds to these observations that tuberculin can produce ocular changes. The possibility that periodic ophthalmia is a hypersensitiveness to some foreign protein has not been disproved. It may well be that the acute stage of the disease is an allergic or hypersensitive type of reaction, and the quiescent period represents a period of desensitization. Schäper[26] is of the opinion that an inherited predisposition to such a hypersensitiveness also exists in periodic ophthalmia. Unfortunately, not enough positive experimental evidence is at hand to establish this theory. Further experimental studies are necessary before we may accept or discard the hypersensitiveness theory.

Parasites.—Various helminthic parasite larvae have been incriminated in periodic ophthalmia. Helminthic larvae have been observed in the anterior chamber of equine eyes upon many occasions. Nicholls and Crawford[34] describe some larvae that were removed from horses' eyes in Ceylon. Schwartz[35] described larvae, which he identified as belonging to the genus Setaria, that were removed surgically from equine eyes in the United States. The symptoms and lesions in these cases were not those of periodic ophthalmia. The larvae were visible grossly in the anterior chamber of the affected eyes. Larvae are not visible in typical periodic ophthalmia cases.

In 1938, Iyer[36] reported some interesting observations from India. He encountered a disease which had all of the characteristic symptoms, the course and many of the lesions of periodic ophthalmia and, in fact, had been called periodic ophthalmia for several years. Histopathologic observations on several acute cases revealed unsheathed larvae in the substantia propia of the cornea and in the lacrimal glands. A marked cellular reaction was present around each larva. Iyer concluded that the larvae were the cause of all of the ocular symptoms. He believed that the disease he studied was a true verminous ophthalmia. The marked similarity of the symptoms and course of the disease described by him to those which we recognize in periodic ophthalmia is noteworthy. In our laboratory complete histopathologic studies of over 80 eyes from cases of periodic ophthalmia in its various stages did not reveal parasites in the ocular structures.

Further, in none of the cases we have studied clinically or pathologically has the cornea been involved, except for some peripheral vascular infiltration into the substantia propria. No lesions in the cornea that would suggest parasite invasion were encountered. Either Iyer was dealing with a disease different from the periodic ophthalmia we recognize here or he encountered two attendant ocular diseases.

In studies on the pathology of periodic ophthalmia we have observed that most cases of the disease exhibit parasitic lesions in organs other than the eye. Lesions

[30]Gmelin, W.: Mondblindheit. Tierheilk. u. Tierzucht, xi (1936), pp. 446-450.

[31]Berrar, M., and Manninger, R.: Untersuchungen über die Ätiologie der Mondblindheit, I. Arch. f. Tierheilk., xlix (1929), pp. 132-158. Manninger, R.: Untersuchungen über die Ätiologie der Mondblindheit, II. Arch. f. Tierheilk., lxi (1930), pp. 144-172.

[32]Guillery, H.: Ueber Fermentwirkung am Auges und ihre Beziehungen zu sympathelischen Ophthalmologie. Arch. f. Augenh., lxviii (1911), p. 242; lxxii (1912), p. 99; lxxiv (1913), p. 132; lxxvi (1914), p. 226. *Ibid.:* Untersuchungen ueber Uveagifte. Arch. f. Augenh., lxxviii (1914), p. 2.

[33]Poos, F.: Ueber die Wirkung des Tuberculins auf normale Augen. Arch. f. Ophthalmol., cxxv (1930), pp. 41-61.

[34]Nicholls, L., and Crawford, M.: Verminous ophthalmia of the horse in Ceylon. Ceylon J. Sci., i (Nov. 18, 1925), pp. 147-149.

[35]Schwartz, B.: Nematodes belonging to the genus Setaria parasitic in the eyes of horses. No. Am. Vet., viii (Feb. 1927), pp. 24-27.

[36]Iyer, P. R. K.: A form of verminous ophthalmia in equines. Indian J. Vet. Sci. & Anim. Husb., viii (March 1938), pp. 3-11.

caused by migrating larvae were noted in the intestinal wall, peritoneum, liver, pancreas, mesenteric artery, lung and, to a limited extent, in other organs. These lesions roughly compared in intensity and age with those seen in the eye. In other words, many new parasitic lesions usually were found in new, acute cases. Old or quiescent cases usually exhibited old, healed parasitic lesions in the visceral organs. In these cases (*vide infra*) it was further noted that a local tissue eosinophilia was present in the organs along the channel of lymph drainage from the parasitic lesions. Numerous collections of eosinophiles were seen constantly in the tissues around the parasites, in the lymph glands and in the spleen. Small foci of small round cells in the liver also were observed. These may have been part of the systemic reaction to the parasites. At least, this observation indicates that there is some product developed locally as a result of parasite invasion of the tissues, which in turn is absorbed into the general circulation. This would suggest that possibly the migration of larvae could have some effect upon the eye through absorption and circulation of a toxin liberated by the larvae. More experimental work will be necessary, however, before this can be established definitely. It also must be kept in mind that parasites are common in most animals and may be present with any one of several other diseases.

Gmelin[30] suggests that adult strongyles and ascarids acting together produce the disease. He visualizes the ascarid producing a toxin that is absorbed through the intestinal wall by way of injuries produced by the biting habits of the strongyles. However, we have autopsied several acute and active cases of periodic ophthalmia in which no ascarids and very few adult

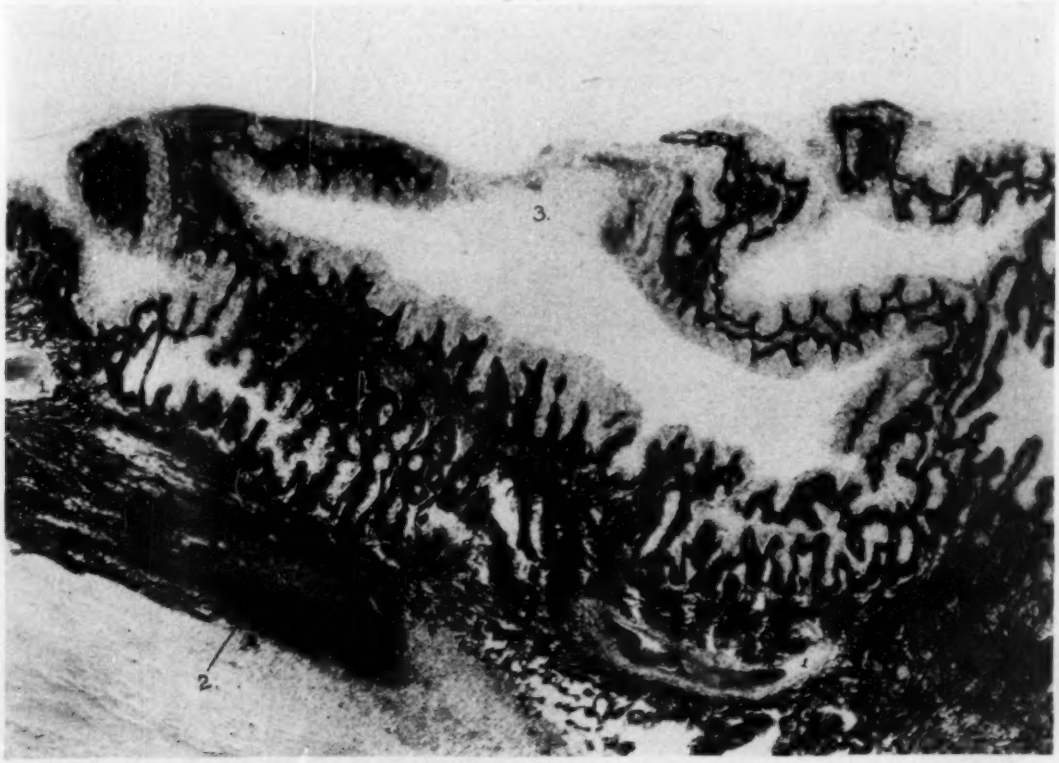

Fig. 12. Acute periodic ophthalmia (x 50)—an enlarged view of ciliary apparatus seen in figure 10. Note congested blood vessels (1), lymphocytes in ciliary body (2), lymphocytes and fibrin exuding from ciliary processes (3).

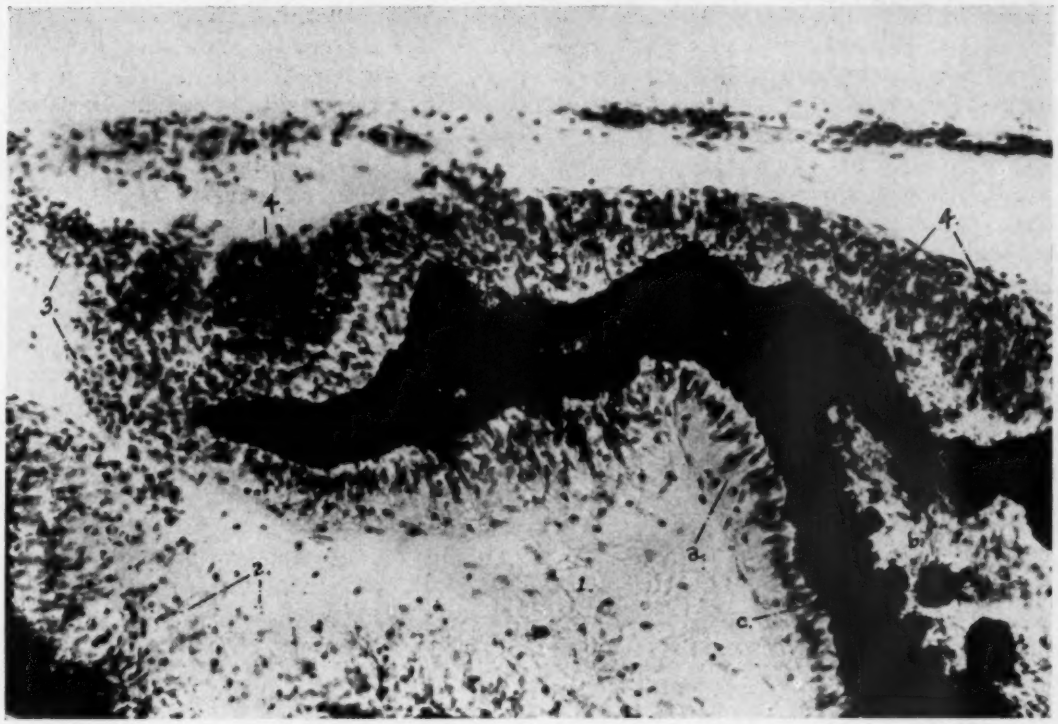

Fig. 13. Acute periodic ophthalmia (x 205)—an enlarged view of ciliary processes seen in figure 10. (a) Epithelium of ciliary process, (b) stroma of ciliary process, (c) uveal pigment of ciliary process, (1) fibrin, (2) loose bits of uveal pigment, (3) lymphocytes free from process, (4) lymphocytes in epithelium of ciliary process.

strongyles were present in the intestinal canal.

Clark[37] noticed a high incidence of intestinal parasites in some horses affected with periodic ophthalmia. He also observed that several cases autopsied had verminous aneurisms or thrombi in the greater mesenteric artery. He suggested that these verminous aneurisms and thrombi may serve as points of origin for emboli which could eventually reach the vascular structures of the eye. Further, these foci may be a means of introducing a parasitic toxin into the blood stream which may eventually reach the eye. Clark mentioned the possibility that parasites or insects may act as vectors of the disease.

To summarize our discussion of the theories as to the etiology of periodic ophthalmia, our present knowledge indicates that known bacteria do not cause the disease. Heredity, thyroid deficiency and nutritional deficiencies are probably of little significance. An ultravirus, hypersensitiveness and parasites, or a combination of the last two, seem to be the most important possibilities at present, although further experimental evidence is needed to establish the etiology of this disease. Until we have more definite information as to the exact cause, our efforts at control and treatment are apt to be unsuccessful.

[37]Clark, H. C.: Moon blindness (periodic ophthalmia), a preliminary report. Vet. Rec., xlix (1937), pp. 1322-1324.

COURSE AND SYMPTOMS

The clinical course is characterized by recurrent acute manifestations separated by quiescent periods. The acute symptoms are essentially those of acute iridocyclitis. Catarrhal conjunctivitis in one or both eyes is usually the first indication of an acute attack (fig. 9). This is accompanied by photophobia and lacrimation, and may be associated with general depression and slight elevation of temperature (1 to 1.5

F.). The temperature returns to normal before the second day. This day of the acute attack is usually the time that the disease is first noticed. Here photophobia is marked and the conjunctiva congested. Frequently a cellular exudate in the anterior chamber is present by the second day (fig. 8). This may be recognized as a yellowish or white flocculent exudate in the anterior chamber, or a diffuse cloudiness of the aqueous. In the former case the exudate gravitates to the ventral part of the chamber, leaving the dorsal part clear. In the latter case a diffuse cloudiness appears on the posterior surface of the cornea. In either case examination of the deeper structures is difficult. The flocculent exudate at this stage consists of lymphocytes, fibrin, serum and occasionally erythrocytes. Hence, although it resembles pus clinically, the condition is not true hypopyon.

Myosis is present early and is characteristic of the disease. Not only is the pupil contracted, but also a paralysis of the muscles of the iris is evidenced by the very slow response to mydriatics. Repeated instillations of atropine over a period of several hours may be necessary to complete dilatation of the pupil. Commonly in recurrent cases, less frequently in new cases, small bits of pigment from the iris adhere to the anterior capsule of the lens. These are visible as dark brown foci on the lens after the pupil is dilated. Early and repeated instillations of mydriatics are necessary to prevent the occurrence of this complication.

The intraocular tension in typical cases is decreased, even during the acute stage. If only one eye is affected, a comparison with the opposite eye confirms this. A practical comparison may be made by digital pressure over the upper eyelids and by noting the characteristic wrinkling of the upper eyelid of the affected eye (fig. 22). A more accurate estimate of the intraocular tension may be secured through the use of a tonometer, such as the type developed by

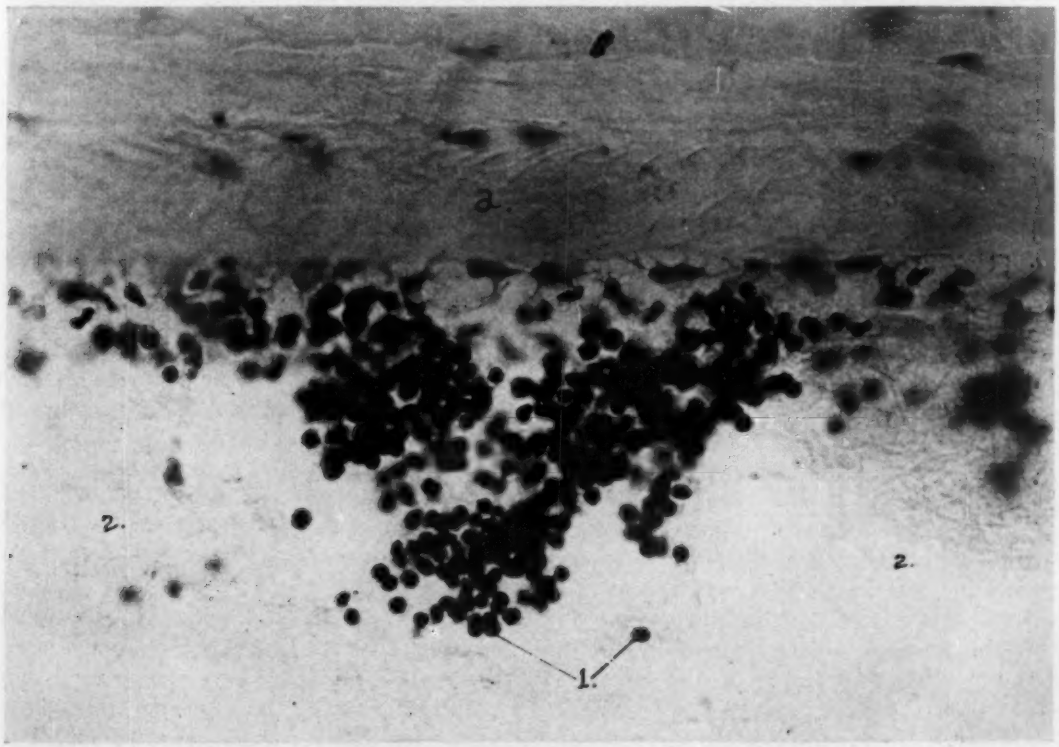

Fig. 14. Acute periodic ophthalmia (x 640)—an enlarged view of cornea and exudate in figure 10. Note that the exudate in the anterior chamber consists almost entirely of lymphocytes (1), serum, and fibrin (2) which in this instance are in contact with the posterior surface of the cornea (a).

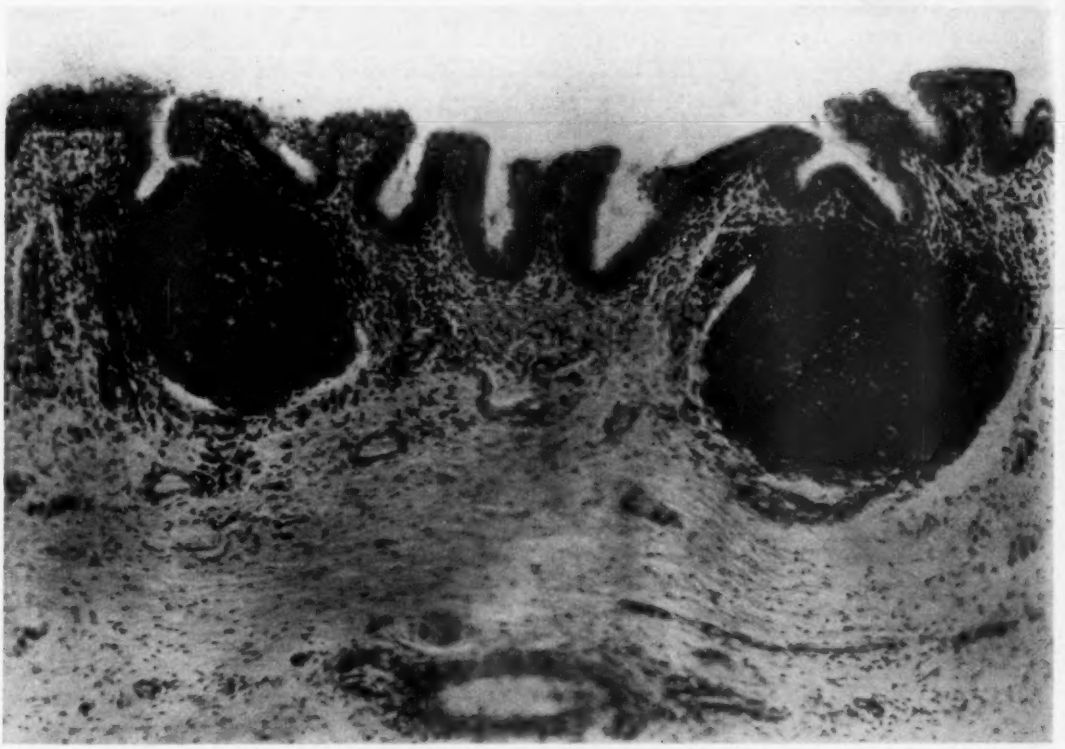

Fig. 15. Lymph nodules in conjunctival stroma (x 42). This type is commonly seen in normal animals.

Schiotz. A local anesthetic should be employed with this instrument. Securing fixation of the head is essential. At present the Schiotz tonometer is not used extensively in examining equine eyes, since it is cumbersome and does not yield positive diagnostic evidence.

Ophthalmoscopic examination during the acute stage is not of much value. Cloudiness of the media makes it difficult or impossible to see deeper than the anterior capsule of the lens. Often, even the anterior surface of the iris is not visible. Slit-lamp examinations have not been described.

Pericorneal vascular infiltration occurs in severe attacks. Usually the blood vessels are first visible on the ventral border of the cornea about the third or fourth day. Gradually these vessels extend toward the center of the cornea. In a few cases they may be visible all around the periphery, but rarely do they extend to the center of the cornea. In a few extremely severe cases the cornea may become concave in its center, due to the decreased pressure in the anterior chamber.

As a rule, at the end of the acute symptoms the exudate in the anterior chamber is absorbed, and the media gradually regains its transparency. The end of the acute course is usually reached in one to three weeks. The clinical picture at this time varies a great deal, depending upon the severity of the attack, number of previous attacks and treatment. A not infrequent occurrence is the appearance of acute symptoms in one eye a few days following abatement of acute symptoms in the opposite eye. Hence, the length of the quiescent period between acute exacerbations may vary greatly. A period of time varying from a few days to over a year may intervene between acute symptoms. Thus, the reappearance of acute symptoms is not regularly spaced. It has, therefore, been suggested that the name recurrent ophthalmia more accurately describes the syndrome than periodic ophthalmia.

In one typical case in a 20-month-old Thoroughbred filly in which the animal was closely observed and accurate records kept, nine acute attacks occurred over a period of 14 months. Both eyes were involved. The first attack occurred in the right eye. It was followed 47 days later by acute symptoms in the same eye and 22 days later by acute symptoms in the left eye. Forty-three days following, both eyes were acutely affected, and 81 days elapsed before the next attack in the left eye. The right eye was again involved in 45 days and in 45 more days the left eye again showed acute symptoms. The last attack occurred in the right eye 74 days following acute symptoms in the left eye. Cataracts were present at this time in both eyes. They were complete and produced total loss of vision in both eyes seven months later. The total elapsed time from the appearance of the initial symptoms to the complete loss of vision was 20 months. The course in this case is quite typical of severe periodic ophthalmia. However, many variations occur.

The quiescent period is characterized by the absence of acute inflammatory symptoms and by the presence of the lesions of the previous acute manifestations. One typical acute attack in many cases leaves some lesions that may be detected upon thorough examination. An ophthalmoscope is essential to detect most of these, although occasionally some may be recognized by the unaided eye. Many of the lesions may be observed in the living subject, others only at autopsy.

Probably the most common lesions in early cases are the exudates in the vitreous. These appear as dark, "pepper-like" bodies through the ophthalmoscope or may produce a diffuse cloudiness of the vitreous which blurs the view of the fundus. These opacities consist of fibrin, serum and lymphocytes. During the quiescent period in some cases, diffuse or localized areas of congestion in the retina are seen with the

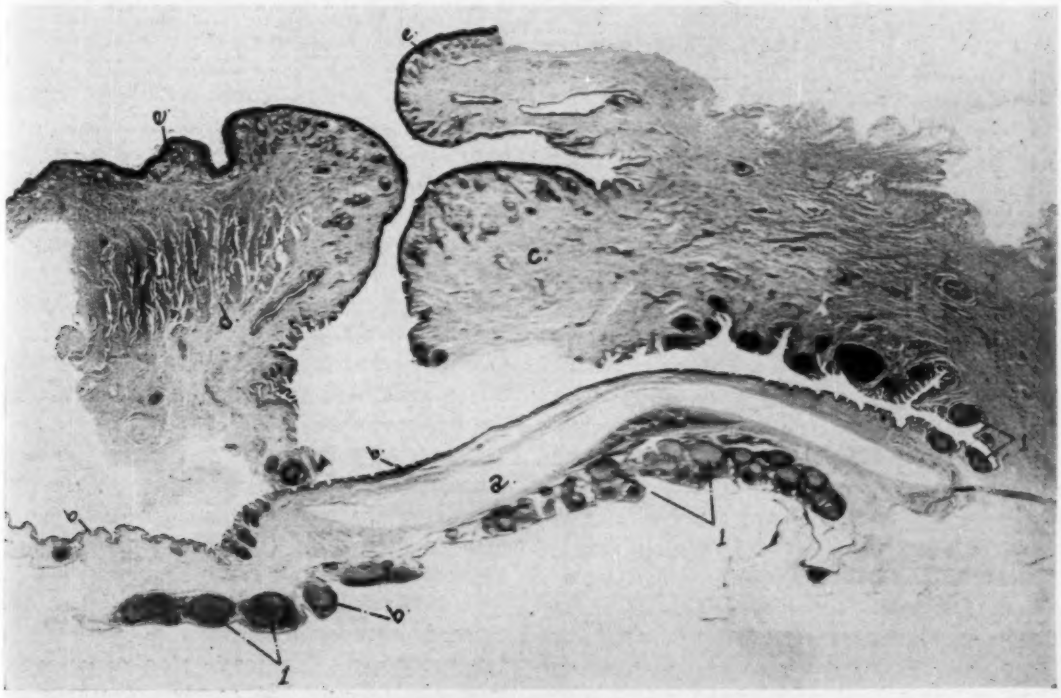

Fig. 16. Quiescent periodic ophthalmia—sagittal section cut near medial canthus to include base of third eyelid (x 7). Note the numerous lymph nodules (1) in the subepithelial stroma of the conjunctiva. (a) Cartilage of third eyelid, (b) conjunctiva, (c) lower eyelid, (d) upper eyelid, (e) skin.

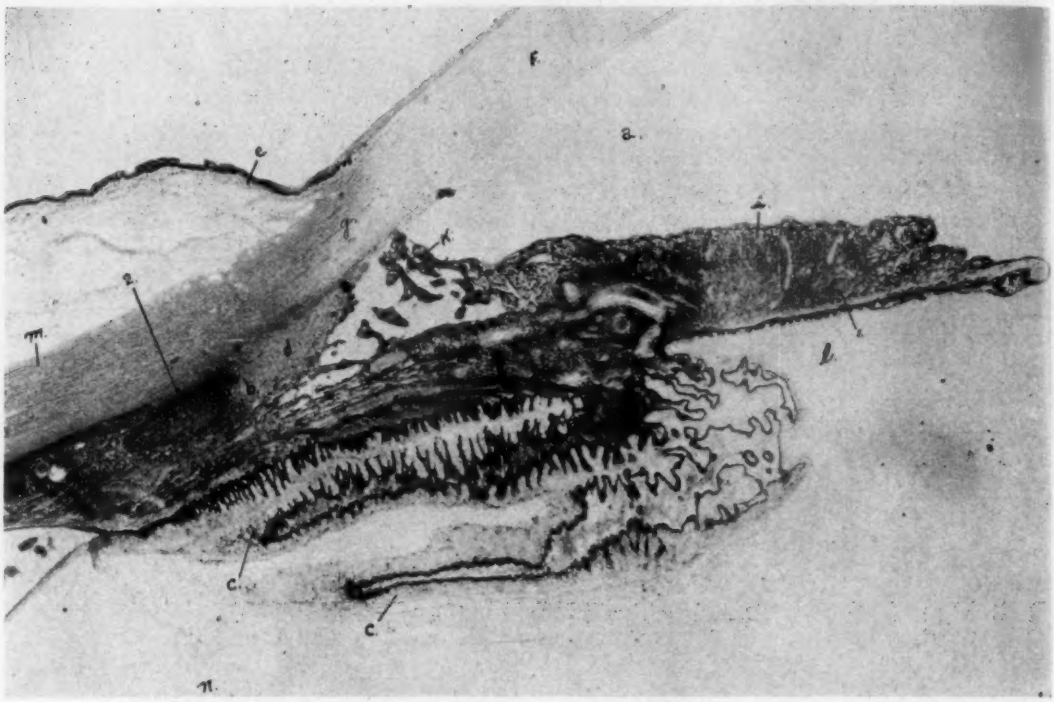

Fig. 17. Quiescent periodic ophthalmia—meridional section through ventral limbus (x 15). Compare with figure 5. Note collections of lymphocytes in iris (1) and ciliary body (2). Also note thickening and adhesions of ciliary processes (c) by fibrin and lymphocytes. Some fibrinous and lymphocytic exudate also may be seen in the vitreous (n).

ophthalmoscope. Although the connection of these lesions in the retina with periodic ophthalmia is not entirely clear, the histopathologic observations in some cases (*vide infra*) indicate that the retinitis may be a late part of the periodic ophthalmia syndrome. Exudates on the lens capsule are common. They may consist of fibrinous deposits on the anterior or posterior capsule, or of iris pigment on the anterior capsule. Cataracts are common sequels of repeated acute attacks. The lens opacity may be partial or complete, but is most commonly complete in old severe cases.

Posterior synechias are common. These may be recognized in the living subject by failure of the iris to respond to mydriatics; or, if the pupil does dilate, deposits of iris may be torn, part of it adhered and part free. In the normal equine eye the pupil has a horizontal slit shape when contracted; when dilated it is smoothly circular. If posterior synechias occur, this circular shape of the dilated pupil is markedly altered In a few cases complete "ring" synechias are seen. In these instances the pupil is markedly contracted, often obliterated, and the iris adheres to the lens around the entire pupillary border. This condition causes the aqueous to collect in the posterior chamber which, in turn, bulges the iris forward into the anterior chamber (iris *bombé*). This may be further complicated by the formation of anterior synechias at the points of contact of the iris and cornea.

Phthisis bulbi with the characteristic wrinkling of the upper eyelid (due to the decreased intraorbital tension) is a common indication of quiescent periodic ophthalmia (fig. 22).

It is Errington's[38] observation that decreased intraocular tension is not only a fairly constant diagnostic sign of periodic ophthalmia, but that marked decrease of tension is also an unfavorable prognostic

[38]Errington, B. J.: Ophthalmology in Equidae. J.A.V.M.A., xcviii (Feb. 1941), pp. 115-123.

sign. He observed that those cases exhibiting marked decrease in tension also manifested the most lesions. Those eyes in which the tension was lowered only slightly, showed fewer intraocular lesions, regardless of the number and severity of previous acute attacks which they had undergone. The amount of exudate in the vitreous seems to be particularly increased by marked decrease in tension.

In old cases chronic degenerative changes become associated with the lesions described above. The eyeball becomes markedly atrophied, the lens densely opaque (often with large blood vessels visible upon it) and the cornea reduced in size and increased in convexity by the shrinking of the globe (fig. 23). Of course, these animals are totally blind in the affected eyes. A further discussion of the lesions will be found under the section headed "Pathology."

DIAGNOSIS

The diagnosis is based upon the symptoms in the acute case or upon the lesions if the case is quiescent and it is confirmed by the history when available. Laboratory tests are not used at present. In initial attacks, or when an accurate history is not available, periodic ophthalmia must be differentiated from changes due to trauma (conjunctivitis, keratitis) and from iritis and iridocyclitis, which are occasionally associated with equine influenza or other febrile conditions. Table I may be of assistance in diagnosis.

TREATMENT

As long as the exact etiology of this disease remains obscure, the treatment can only be symptomatic and empirical. Both general and local methods of treatment have been tried. The production of nonspecific protein reactions by the subcutaneous or intravenous injections of typhoid vaccine or sterile milk is believed to be of value by some investigators in shortening the course of the acute disease, but none has been constantly successful in preventing recurrences of the acute symptoms. These injections are not without danger of serious shock and their therapeutic value is questionable. Numerous other drugs have been used for their systemic effect. Among them are neoarsphenamine, mercuric iodide, potassium iodide, sodium iodide, atoxyl, sulfanilamide, biniodide of quinine, and quinine. None of these has constant proved merit. Injections of Lugol's solution into the periorbital fat have been tried without success.

Local treatment of the affected eye is the second form of therapy in this disease.

TABLE I

	PERIODIC OPHTHALMIA	INFLUENZA	TRAUMATA
Eyes affected	One or both	One or both	One usually
Photophobia	Marked	Moderate	Mild to severe
Eyelids	Little swelling; may be wrinkled	Edematous	Edema if injured
Lacrimal discharge	Serous	Serous	Serous to purulent
Conjunctiva	Marked hyperemia	Yellowish, edematous, congested	Edematous and inflamed, depending upon injury
Febrile reaction	None, or slight 1st day	Marked for several days	None usually
Cornea	Marked pericorneal injection; anterior surface usually not opaque	Usually no marked pericorneal injection	Cornea opaque on anterior surface at site of injury
Pupil	Contracted; difficult to dilate	Moderately contracted; dilates slowly	Normal
Aqueous	Cloudy, white to yellowish flocculent exudate in ventral part in 2 to 4 days or longer	May have fibrinous exudate in aqueous	No exudate in anterior chamber unless have penetration
Intraocular tension	Decreased	May be decreased	Normal
Iris	Discolored; markings indistinct	May be discolored	Normal

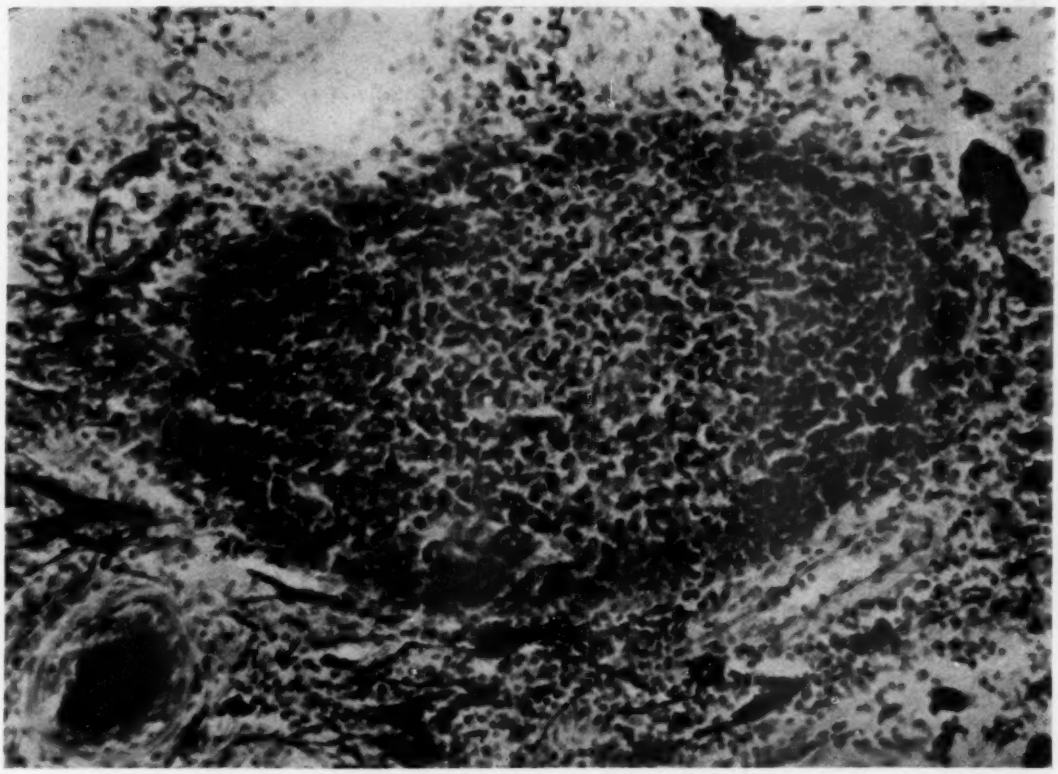

Fig. 18. Quiescent periodic ophthalmia—circumscribed focus of lymphocytes in ciliary body (x 235).

Early and repeated instillations of a mydriatic, such as atropine sulfate (2%), is the most rational procedure. Instillations every 20 minutes until the pupil is dilated, then every 2 to 4 hours to keep it dilated, are advisable. Prompt dilatation of the pupil is essential to prevent the formation of synechias and related lesions. However, mydriatics can not be expected to prevent exudation into the vitreous or the degenerative changes in the lens following repeated attacks. Warm packs tied over the affected eye, and placing the animal in a darkened stall, are desirable. Instillation of butyn solution (1%) into the eye is of value in reducing the acute pain. The best that can be said for the treatment outlined above is that it should prevent the formation of synechias and the associated lesions in the iris. It does not affect the exudation into the vitreous, nor does it prevent the recurrence of the acute symptoms. Myotics, such as eserine, are contraindicated.

The discouraging status of the therapeutic methods tried in periodic ophthalmia emphasizes the imperative need for more precise knowledge relative to the etiology of this disease.

PATHOLOGY

In describing the pathologic lesions of periodic ophthalmia it is desirable to classify the disease into three stages. As the clinical manifestations are readily divided into acute, quiescent and old (*vide supra*), so are the pathologic lesions most satisfactorily studied under these three classifications.

An acute case is one in which the active recurrent inflammatory symptoms are present in the eye.

In a quiescent case the acute symptoms are in abatement, but may reappear at any time.

An old case is one in which the acute symptoms have entirely ceased to appear.

It is difficult to predict when the active disease has finally ceased, but any case that has shown no acute symptoms for more than a year and presents the typical chronic degenerative changes, we arbitrarily classify as old. Quiescent and old cases exhibit the pathologic lesions remaining from previous acute manifestations

plus any secondary degenerative or inflammatory changes that may have supervened.

The pathologic lesions of periodic ophthalmia have been described by Jakob,[6] Schlotthauer[39] and a few others. Most investigators, however, have limited their descriptions to the lesions in the eye following repeated attacks. We have been fortunate in securing eyes from animals in the earliest stages of the disease as well as in all subsequent stages. The pathologic changes recorded herein are for the most part based upon our observations on 39 autopsies of periodic ophthalmia cases. As most of these cases were bilateral, this represents over 70 eyes affected with the disease. Over 30 normal eyes also were studied histologically. Among the diseased eyes, 18 were in the acute stage, 32 in the quiescent stage and 24 were classified as old.

Ocular Lesions in Acute Cases.—Grossly the pathologic lesions observed in an acute case may be closely correlated with the clinical manifestations observed during the same period. However, some of the lesions are not observed until autopsy and removal of the ocular structures. At autopsy the conjunctival hyperemia, discoloration of the iris, contraction of the pupil, new blood vessels at the periphery of the cornea and exudates in the anterior chamber are first observed. The iris is frequently mottled with yellowish discolorations. The exudate in the anterior chamber may produce a diffuse translucency in the aqueous humor or may be seen as a yellowish flocculent exudate which gravitates to the ventral part of the chamber. The latter type of exudate, which is most common, appears to be fibrinous and cellular and may include small, loose bits of uveal pigment from the iris. The new corneal vessels are slender and extend in a radial direction from the limbus toward the centrum of the cornea. They are most in evidence in the ventral half of the cornea, but may extend all around the periphery.

When the globe is opened (after fixation in formalin) the lesions in the anterior chamber can be clearly seen. In addition, fibrinous exudate is recognizable over the surface of the ciliary body and ciliary processes as far back as the ora ciliaris retinae* and may extend over the posterior lens capsule. Posterior synechias may be present. Small bits of pigment from

[39]Schlotthauer, C. F.: Recurrent ophthalmia. No. Am. Vet., xiv (June 1933), pp. 18-29. *Ibid.:* Recurrent ophthalmia (iridocyclitis). Vet. Med., xxxiii (1938), pp. 210-215.

*Ora serrata in the eye of man.

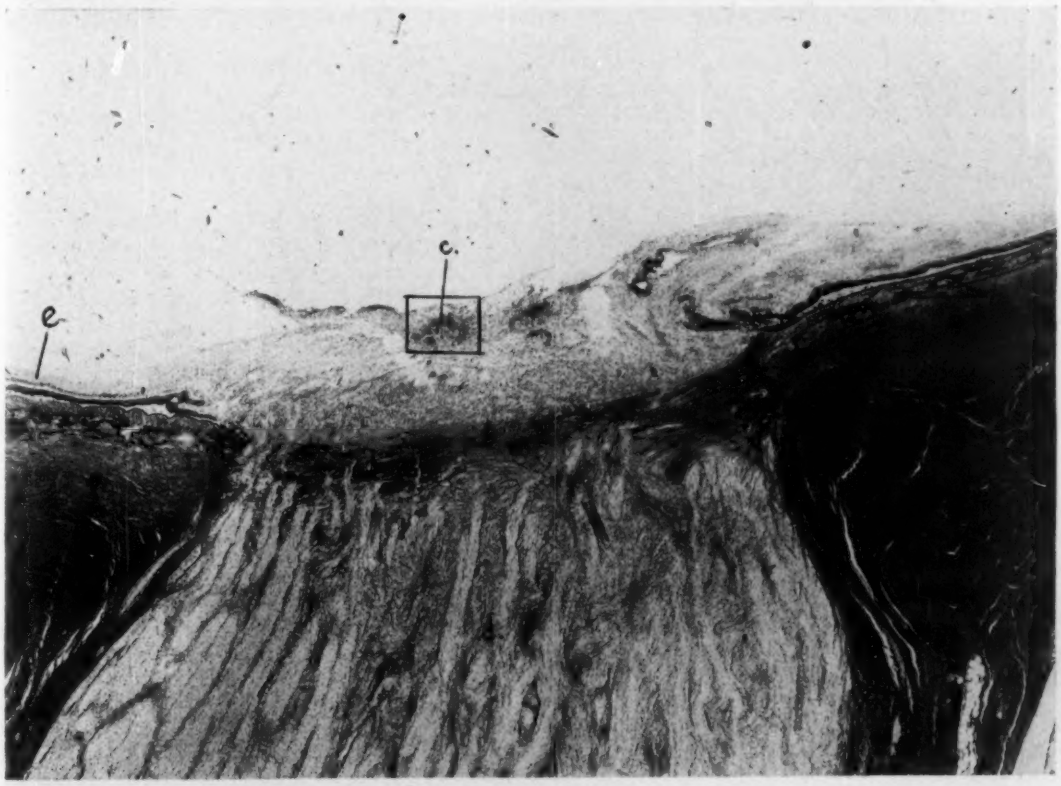

Fig. 19. Quiescent periodic ophthalmia—optic papillus (x 20). See figure 20 for higher magnification.

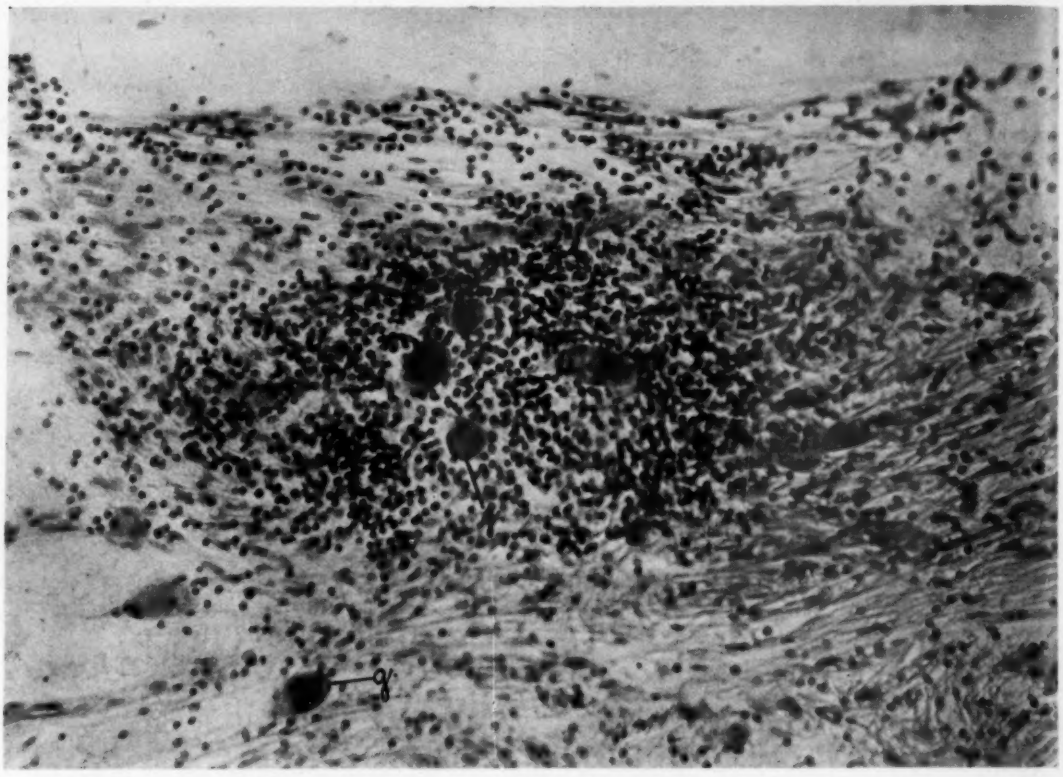

Fig. 20. Quiescent periodic ophthalmia—enlarged view (x 280) of optic papillus (c) in figure 19.

the iris often are seen adhering to the anterior lens capsule. Early capsular cataract may be in evidence. A varying amount of exudate may be seen by the unaided eye in the vitreous body. Numerous tiny, opaque foci and fibrinous strands may be visible in the anterior part of the vitreous; usually no gross lesions are evident in the acute case in those parts of the globe posterior to the ora ciliaris retinae.

Histopathologically the lesions in the acute cases usually are limited to the structures in which the gross lesions are seen. Aside from the few new capillaries in the substantia propria near the scleral border, the cornea is quite constantly free from pathologic involvement. The exudate in the anterior chamber (fig. 10) is seen to consist of lymphocytes, fibrin and serum. A similar exudate usually is visible in the stroma and on the surface of the iris, in the posterior chamber and on the lens capsule. It is extensive in the ciliary processes (fig. 13) and cells may be seen exuding into the vitreous. The iris and ciliary body are extensively infiltrated by lymphocytes. The blood vessels of the anterior uvea are highly congested. The spaces of Fontana may be filled with serous exudate. Lymphocytes, fibrin and serum along with a few monocytes and plasma cells are visible in the vitreous. Commonly, the anterior part of the chorioid also is invaded by lymphocytes.

The retina appears to remain remarkably free from involvement in the early acute stages. Aside from contact with the exudates free in the vitreous, the retina is not affected until several acute attacks of the disease have occurred. Since the histopathologic changes that do occur later in the retina appear to be secondary, we shall describe them under the discussion of the quiescent and old stages of the disease.

Lesions in Accessory Ocular Structures, Acute Stage.—Grossly the conjunctiva of the eyelids is congested and may exhibit small, white, round foci 1 to 2 mm. in diameter, especially on the bulbar surface of the membrana nictitans. This congestion also is observable microscopically. In the acute stage large conglomerate collections of lymphocytes are visible in the conjunctival epithelium (fig. 16). They are especially large and numerous on the bulbar surface of the membrana nictitans. These collections of cells usually have the appearance of lymph follicles, but frequently are loosely scattered through the conjunctival stroma. Very often small foci of eosinophiles are visible in the stroma a short distance from the lymphoid

nodules. Occasionally lymphocytes have migrated through the epithelium of the conjunctiva. These lymph nodules are also present in the vicinity of some of the large tear ducts. Sometimes lymphocytes will be visible in the lumen of the duct as well.

Lymph follicles are also commonly, but not constantly, present in the conjunctival stroma of horses showing no other ocular lesions (fig. 15). We also have observed them in the conjunctiva of newborn foals and in fetuses. In normal eyes, however, they appear to be fewer in number, smaller in size and arranged in a more orderly manner. Figure 15 illustrates the common appearance of the latter nodules. The mere presence of lymphoid nodules in the conjunctival stroma of the equine eye is probably not indicative of periodic ophthalmia. However, hyperplasia and moderate disorganization of these lymphoid elements does appear to be a part of the periodic ophthalmia syndrome.

The skin of the eyelids, the Meibomian glands, the suderiferous and sebaceous glands of the eyelid, and the gland of Harder remain free of pathologic involvement.

To summarize, it is evident that acute periodic ophthalmia is primarily an iridocyclitis, characterized by congestion and lymphocytic infiltration of the iris and ciliary apparatus with extravasation of fibrinous, serous and lymphocytic exudates into the anterior and posterior chambers and vitreous body. Simultaneous congestion and lymphocytic infiltration of the conjunctiva also occur.

Ocular Lesions in Quiescent Stage.—As has been discussed above, the lesions present during the quiescent stage are those which are left as a result of previous acute manifestations. Logically, the extent of the lesions present in this stage is dependent upon the number and severity of previous acute attacks of the disease.

Grossly the lesions customarily seen in quiescent cases following one or two acute attacks are not extensive and may easily be overlooked. Exudates in the vitreous, bits of iris pigment on the anterior lens capsule, partial posterior synechias, small tears in the iris, irregularities in the shape of the pupillary margin, or small mottled areas in the iris may be all that can be observed grossly. In cases of longer standing the extent of these lesions may be considerably increased and, in addition, lens opacities of various degrees may be present. It is possible for an eye to lose all of its vision and still have further acute attacks of the disease.

Histopathologically the lesions in the quies-

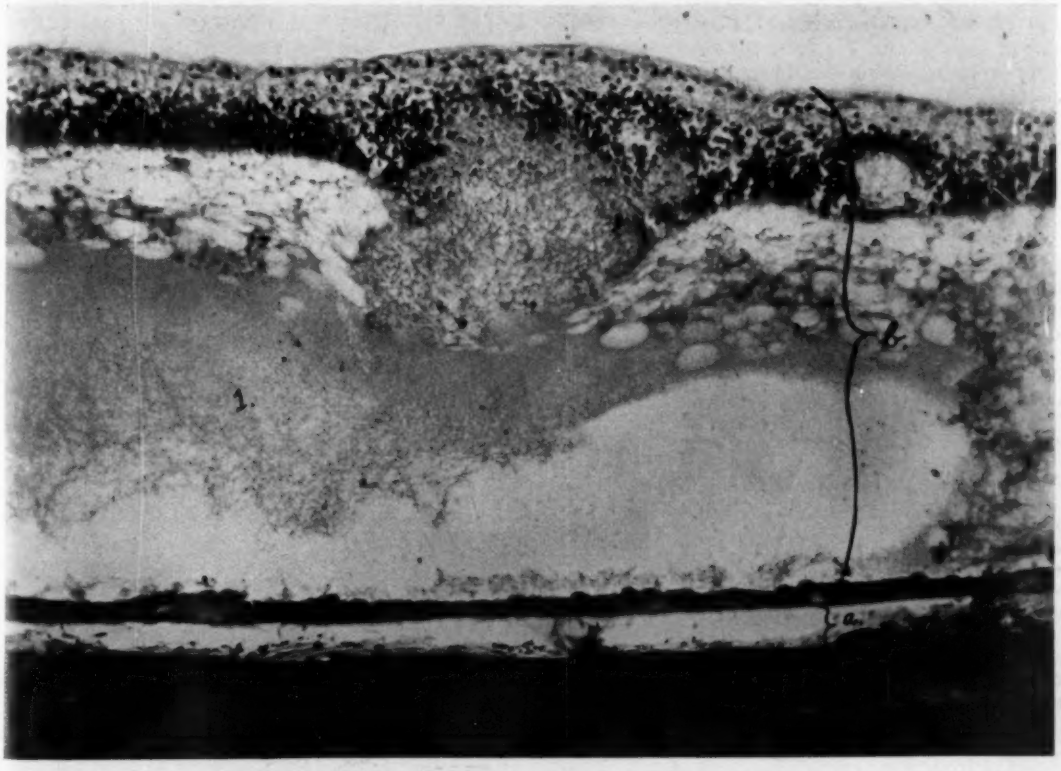

Fig. 21. Late quiescent periodic ophthalmia—retina, chorioid and sclera (x 140). Compare with figure 6. Note thickening, detachment and disorganization of retinal layers. (a) Chorioid, (b) retina, (c) sclera, (1) serous exudate in retina.

Fig. 22. Old periodic ophthalmia. A cataract is seen through the irregular-shaped pupil. Note the characteristic wrinkling of the upper eyelid caused by decreased intraorbital tension.

cent stage are characteristic. In the early case the lesions are limited to the iris and ciliary body. The presence of circumscribed lymphoid nodules in the iris and ciliary body is readily noticeable. These nodules vary in size and are usually closely related to small blood vessels (figs. 17 and 18). They are not present in normal equine eyes, but are quite constantly present in eyes affected with periodic ophthalmia. They vary in size a great deal. Some may be visible grossly in the stained section. Small collections of these lymphocytes may be seen occasionally around capillaries in the optic papillus (fig. 20). These are frequently, but not constantly, present in this location.

Thickening of the epithelium of the ciliary processes with fibrin and lymphocytes (fig. 17) is also quite constant. The blood vessels of the anterior chorioid are numerous and thick-walled. Lymphocytes, fibrin and serum are commonly present in the vitreous. Often the lymphocytes are clumped together—these are probably the small opaque masses seen with the ophthalmoscope.

Fibrinous and lymphocytic exudates are often present on the lens capsules. Commonly the capsule itself may be thickened. Bits of iris pigment occasionally may be seen adhering to the anterior lens capsule. Frequently posterior synechia is also present.

Sometimes microscopic perivascular lymphocytic infiltrations are seen in the optic papillus (figs. 19 and 20). These may be present even though inflammatory changes are not evident in the retina. This suggests that the etiologic factor is borne by the blood stream.

After repeated acute attacks over a long period of time, exudative and degenerative changes in the retina appear. Serofibrinous exudates into the retina between the bacillary layer (rods and cones) and the pigment layer make their appearance. This exudate produces retinal detachment, although the pigment layer of the retina usually remains attached to the chorioid (fig. 21).

It is probable that a low-grade inflammatory reaction is in progress in these late quiescent cases. This would explain the presence of the

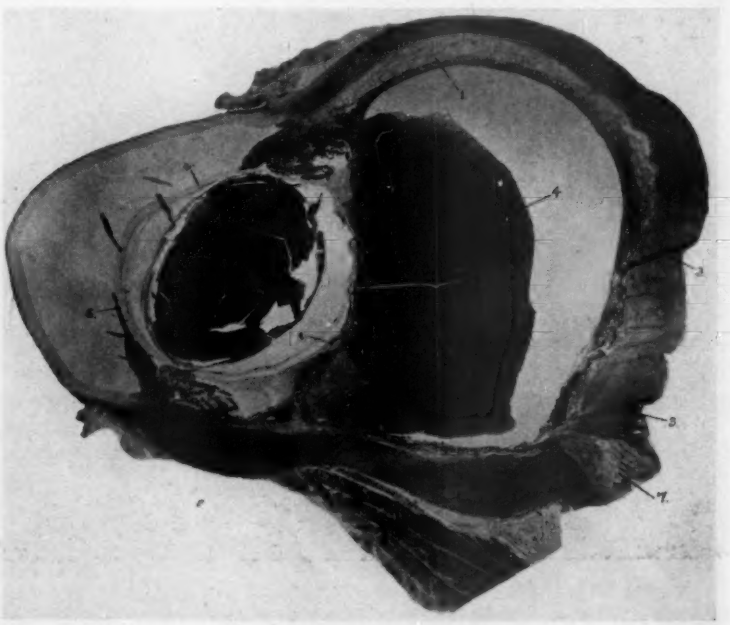

Fig. 23. Old periodic ophthalmia—enlarged photograph of a histopathologic section. Note the marked thickening of the sclera and chorioid, the shrinking of the globe and increased convexity of the cornea. Except for the folds in the celloidin section at (3), the parts in this meridional section are in approximately the same position as they were when the eye was removed at autopsy. Note the edema in the sclera and chorioid (1), the thickened lens capsule (2), the serous exudate (4) which has displaced the retina forward (5) and the posterior synechia (6).

extensive chronic inflammatory changes in old cases.

Ocular Lesions in Old Cases.—The pathologic lesions present in this stage of periodic ophthalmia have been well described. This stage represents the end-point usually reached following many acute attacks of the disease.

Here the pathologic changes are marked and vision usually is completely lost (figs. 23 and 24). Grossly marked phthisis bulbi is first noticeable. The shrinkage of the globe results in increased convexity of the cornea, thickening of the sclera and a characteristic wrinkling of the upper eyelid. The eye loses its full and glossy appearance. Capsular cataracts are common. Often calcareous deposits occur in the lens capsule. Posterior and less often anterior synechias are present. Anterior or posterior displacement of the lens may occur (fig. 24). Usually a thick, organized fibrinous exudate surrounds the lens. Rather large blood vessels may develop on the lens capsule. The iris is frequently torn and mottled with yellowish discolorations. The chorioid is markedly thickened and filled with thick-walled blood vessels. The sclera also is thickened. The ciliary apparatus is thickened and filled with lymphocytes and organized exudate.

The retina commonly becomes detached due to the exudates into it and is displaced forward—often into contact with the posterior lens capsule. It remains attached at the optic papillus and ora ciliaris retinae, however. The serous exudate which produces the retinal detachment occurs between the layer of rods and cones and the pigment layer of the retina. This exudate commonly displaces the entire vitreous body. The retina itself becomes markedly degenerated and frequently only a vestige of connective tissue elements remains (fig. 23).

The cornea usually is involved only due to shrinkage of the bulb and the resultant increased convexity. Vascularization of the substantia propria of the cornea may occur. The optic nerve becomes atrophic.

In summarizing the pathologic lesions it is apparent that the disease is primarily a recurring acute fibrino-lymphocytic iridocyclitis which after repeated occurrences finally results in chronic degenerative and inflammatory changes in most of the internal structures of the eye. The fact that the vascular structures are first and most severely affected indicates that the etiologic factor is blood-borne. The occurrence of perivascular lymphocytic foci simultaneously in noncontiguous parts of the eyeball (*i.e.*, iris and optic papillus) tends to support this tentative conclusion.

Lesions in Other Organs of the Body.—In all of the cases that we have studied pathologically, we have made a thorough search of the body for gross or microscopic lesions that could possibly be correlated with those which occur in the eye. It is difficult to evaluate the significance of any such lesions when found, especially when exact etiologic factors are unknown. It is important to bear in mind also that two or more pathologic processes wholely unrelated,

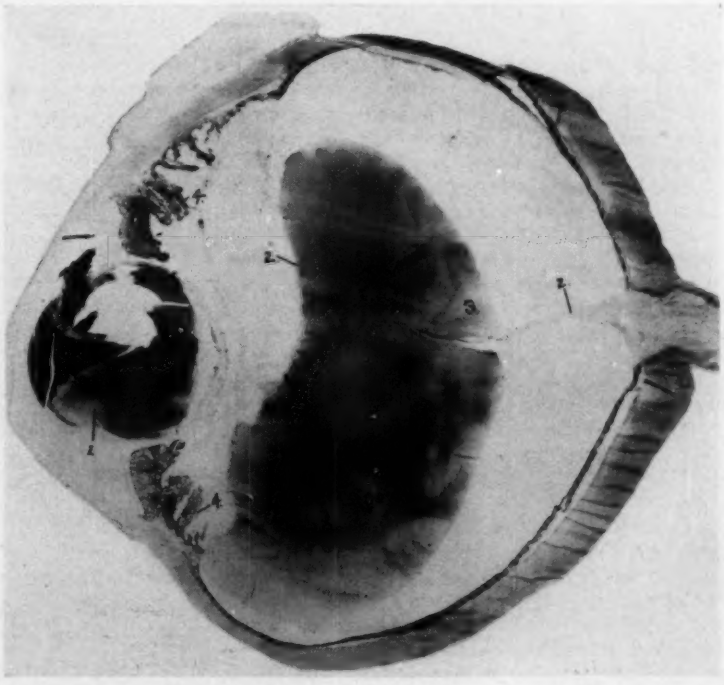

Fig. 24. Old periodic ophthalmia—enlarged photograph of a histopathologic section. Note the anterior displacement of the lens (1), remnant of the retina (2), the serous exudate which has displaced the retina forward (3) and the adhesions and thickening of the ciliary processes (4). Part of the lens was broken out in cutting the section.

may be present in the same animal at one time.

In all of the acute cases that we have studied, and in some quiescent ones, we have noted what we consider a marked parasitic reaction in many organs. This reaction is evidenced by extensive eosinophilic infiltration in the submucosa of the intestines, mesenteric lymph glands, spleen and liver. In some of the organs the eosinophilia was associated with focal

Fig. 25. Complete cataract—right eye of a mule. Note that the pupillary margin is irregular. Also note the deep wrinkle in the upper lid. The diffuse light area above the pupil is the reflex on the cornea of the light used in preparing the photograph and should be disregarded.

lymphoid collections, especially around small blood vessels. Masses of new granulation tissue filled with capillaries, erythrocytes, lymphocytes and eosinophiles were seen under the serosa of the small intestine (ileum especially). In one stallion these were also noted in the testicle and epididymus. Many of the mesenteric lymph glands were edematous and massively infiltrated by eosinophiles. Strangely, these lesions did not seem to be dependent upon heavy intestinal parasitism. On the contrary, adult helminth parasites often were very few in number in the intestinal lumen. It is probable that the lesions described above were produced by migrating larvae (strongyles, ascarids, etc.) and the absorption of toxins from them.

As mentioned under our discussion of etiology the parasitic lesions quite constantly appeared to correspond in extent and stage of development with the lesions in the eye. The new, acute case of periodic ophthalmia frequently had many new, acute parasitic lesions in the visceral organs. Edematous and eosinophilic infiltration of the lymph nodes which drain the structures containing these parasites as well as focal collections of eosinophiles in such places as the spleen and conjunctival stroma strongly suggest that a toxin is liberated by these parasites which, in turn, is absorbed into the general circulation.

This was the only even partially constant pathologic reaction noted that possibly could be correlated with the acute ocular symptoms. It would be jumping at conclusions indeed to state that the parasitic reactions are etiologically related to the ocular lesions. The relatively small number of cases involved and the lack of extensive experimental work precludes the drawing of any conclusions in this regard. However, we believe that these observations should be recorded.

SUMMARY

Periodic ophthalmia is described as a recurrent lymphocytic iridocyclitis of Equidae characterized by acute, quiescent and old stages. The disease is discussed from the standpoint of its epizoötiology, etiology, course and symptoms, diagnosis, treatment and pathology. It usually occurs as a slow-spreading enzoötic; however, sudden and severe outbreaks also are reported. Ophthalmology as it applies to veterinary medicine is discussed. The use of the ophthalmoscope is especially stressed. The various theories as to the etiology of periodic ophthalmia are discussed. It is stated that known bacteria, heredity, thyroid and nutritional deficiencies are probably of little etiologic significance. An ultravirus, hypersensitiveness, parasites, or a combination of the last two seems to be the most valid etiologic possibility in the light of our present knowledge.

The symptoms of the acute stage are described as photophobia, excess lacrimation, myosis, exudate in the anterior chamber, ptosis and decreased intraorbital tension. The course of the acute stage is characterized by sudden onset, acute symptoms lasting one to three weeks, and recurrences after greatly varied lengths of time. The quiescent stage is marked by absence of acute symptoms and the presence of lesions of previous attacks. Such lesions are posterior synechias, capsular cataracts, opacities in the vitreous body and tears or discolorization in the iris. Old cases present chronic degenerative changes in the lens, vitreous and retina. Most common of these

are cataracts, vitreous opacities and retinal detachment.

Treatment is described for the acute stage. This is limited to symptomatic treatment of the affected eye and nonspecific protein-reaction therapy. The best available treatment may shorten the course of the acute attack and prevent many of the common sequels, but no effect upon the recurrence of symptoms can be expected.

The gross and microscopic lesions in all stages of the disease are described and discussed in detail. The lesions in the acute case are limited for the most part to the iris and ciliary body.

Grossly a white, fibrinous exudate usually is seen over the ciliary body and processes. A white, flocculent exudate can be seen in the anterior chamber. Microscopically these exudates are seen to consist of lymphocytes, serum and fibrin. In the early quiescent case few lesions can be seen with the naked eye. Microscopically accumulations of round cells in the iris and ciliary body are constantly seen. In the quiescent case the lesions are those remaining as a result of previous acute attacks and any degenerative or inflammatory changes that may have supervened. In old, long-standing cases chronic inflammatory and degenerative changes may involve all of the internal structures of the eye. Posterior synechias, cataracts, retinal detachment and lens displacement are commonly observed following many acute attacks of the disease.

ACKNOWLEDGMENTS

The assistance of the curator of the U. S. Army Medical Museum, Lt. Colonel James E. Ash, Medical Corps, in the preparation of photomicrographs is gratefully acknowledged. The coöperation of Lt. Colonel Lloyd C. Ewen and Captain Wm. E. Jennings, Veterinary Corps, in the study of clinical material was of distinct aid in this work. I also wish to thank Staff Sergeant Charles L. Barton, Medical Department (Veterinary Service), for his valuable technical assistance in the preparation of histopathologic sections, and Dr. W. Graham Love for help in connection with the bibliography.

Control of the Hog-Kidney Worm (Stephanurus Dentatus)*

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DURING RECENT YEARS, swine-kidney worms have been causing increased losses to swine growers in the eastern part of Virginia. This loss is due not so much to the loss in weight of affected hogs as it is to the condemnation by meat inspectors of livers, kidneys and certain parts of the loin containing these parasites. As a result, packers now discriminate against hogs raised in certain sections where these parasites are known to exist.

The U. S. Bureau of Animal Industry studied the control of hog-kidney worms by certain experiments performed in Georgia. From this work[1] it was concluded that the infestation could be largely prevented by an intricate system of management. The system consists chiefly of protecting the pigs from becoming infested while they are with the sows. After the pigs are weaned, they can be protected readily by being kept on temporary pastures especially prepared for them, and away from the sows and all other swine except pigs from other litters of about the same age which also have been raised under this system. Protection is afforded the pigs during the suckling period by placing pregnant sows, shortly before they farrow, on a pasture which has been sown to a suitable forage crop. There is a bare area all around the pasture about 5 ft. wide on three sides and about 30 ft. wide at one end. Strict adherence to these dimensions is not essential. The important point is to provide a bare strip all around the field and at one end an area more than sufficiently wide to accommodate the shelter houses, water barrel, creep for the pigs and feeding pen for the sows.

From this description it will be noted that the procedure would be difficult for farmers to carry out and at the same time continue with the system of crop rotation which they already are practicing. The section of Virginia where most of the damage is done by these parasites is known as the "peanut section." In this area many of the swine growers make a practice of turning their hogs on peanut fields after the crop has been harvested. Most of them use soybeans and corn to supplement the peanuts. Generally, however, it is the practice to run all the hogs together on the various fields so that practically every part of the farm is occupied by the hogs at some time during the year. In this way the entire farm usually becomes infested with parasites.

Some of these farmers as well as the county agents in several of the involved counties along with the packers in this area requested that this station attempt to devise a method of raising hogs which would be less difficult for farmers to follow than the method recommended by the U. S. Bureau of Animal Industry based on work done at Moultrie, Ga. Accordingly, modified experiments were set up on two farms in that locality.

REVIEW OF LITERATURE

A considerable amount of work has been done on the biology of kidney-worm larvae wherefrom suggestions for the control of the parasite have been made. Ross and Kauzal[2] recommended sanitary measures, such as clean pens and yards, together with the employment of certain chemicals, such as 5 per cent kerol, for the treatment of pens and yards, and the use of well-drained fields exposed to a maximum amount of sunlight. Recommendations by Schwartz[1] and Spindler[3, 4] for the control of

*From the Section of Zoölogy and Animal Pathology, Virginia Agricultural Experiment Station.

[1]Schwartz, B.: Internal parasites of swine. USDA Farmer's Bul. 1787 (1937).

[2]Ross, I. C., and Kauzal, G.: The life cycle of *Stephanurus dentatus* Deising, 1839, the kidney worm of pigs. Australian Counc. Sci. & Ind. Res., Bul. 58.

[3]Spindler, L. A.: Field and laboratory studies on the behavior of the larvae of the swine kidney worm, *Stephanurus dentatus*. USDA Bul. 405 (1934).

[4]*Ibid.*: Skin penetration experiments with the infective larvae of *Stephanurus dentatus*. N. Am. Vet., xv (1934), pp. 32-36.

Stephanurus dentatus based on extensive studies of its life history and the behavior of the larvae emphasized the use of sunlight and clean pastures. Obviously, these measures are positive factors in the control of kidney-worm infection, but frequently the details required make these methods impracticable for farmers to carry out.

It was shown by DeEds[5, 6] that following phenothiazine treatments phenothiazine, thionol and leucothionol were excreted in the urine and bile of man and other animals.

Lipson[7] reported that 10 per cent of the original dose of phenothiazine was recovered from the urine of sheep treated with this chemical and that "Thionol and phenothiazone are excreted mainly in the form of leuco bases which are oxidized to the parent dye stuffs on allowing urine to stand in air."

From the results reported above, it may be assumed that similar products are present in the feces and urine of hogs treated with phenothiazine as described in this study, since the urine voided by these animals within four hours after treatment displayed the familiar color characteristic of that from phenothiazine-treated animals recorded by other workers.

FIELD EXPERIMENTS

In these experiments 49 pigs were raised according to a modified BAI plan, based on the work in Georgia, and 51 pigs were raised according to the methods used generally in that section, to serve as controls. The modification in one case consisted of allowing the sows to farrow in the pasture instead of in individual houses located on a bare area. In the other case, no bare area was allowed on three sides of the pasture. Only a bare area was allowed at the end of the pasture for the houses, feeders and creep. At the close of the experiment all hogs were examined for the presence of parasites, as well as for damage done to such organs and structures as the liver, kidneys and loins.

In general, there was a reduction in the damage done by kidney worms in the hogs raised under this modified plan as compared with those raised in the usual way in that section, but in both cases there was still considerable damage due to these parasites. Several of the hogs were affected, although not extensively. From these experiments we concluded that this is not a completely effective way to prevent infection by kidney worms. It was evident that there was some reduction in the number of parasites present as well as in the damage done by them, but the results obtained did not appear to justify the extra expense and labor involved.

It was considered important to learn what might be accomplished by daily feeding of small amounts of phenothiazine in the ration. Accordingly, one experiment was set up with 25 pigs which each received from 0.5 to 1 Gm. of phenothiazine daily in their feed over a period of five months. An equal number of similar pigs, receiving the same ration but no phenothiazine, were handled under similar conditions for the same length of time and all were examined at the time of slaughter, when they were approximately 7 months of age. It is generally believed that there is no treatment which will have any effect upon kidney worms. It was reasoned, however, that where the larvae reach the liver *via* the portal vein, small amounts of this drug or its oxidation products might kill the larvae at this stage. The results, however, as far as kidney worms were concerned, were not encouraging. At the conclusion of this experiment only 4 of the 25 pigs receiving phenothiazine had died, and in the 21 remaining, which were slaughtered, 28 per cent of the livers were condemned; 9 of the 25 control pigs had died, and in the 16 remaining, which were slaughtered, 87 per cent of the livers were condemned.

It was not possible for us to determine the cause of death in these pigs in each instance, but it was found that several other parasites were present, such as ascarids, lung worms and stomach worms. It was concluded that the effect of the phenothiazine in destroying the adult ascarids and other species of parasites of the ali-

[5]DeEds, F., Eddy, C. W., and Thomas, J. O.: Studies on phenothiazine: V. Fate of phenothiazine in the body. J. Phar. & Exp. Therap., lxiv (1938), p. 250.

[6]DeEds, F., and Thomas, J. O.: Studies on phenothiazine. IV. The biliary excretion and anthelmintic action of thionol. J. Parasitol., xxvii (1941), pp. 143-151.

[7]Lipson, M.: An examination of the urine of sheep dosed with phenothiazine. Australian Counc. Sci. & Ind. Res. (1940), pp. 269-272.

mentary tract[8] was responsible for the better results in the treated hogs.

LABORATORY EXPERIMENTS

In addition to the field work done, certain laboratory experiments were set up to determine what effect larger doses of phenothiazine might have on kidney worms as well as what effect it might have on the host animal.

For this purpose three hogs, designated as 1, 2 and 3, approximately 3 months old and weighing between 45 and 50 lb. each, were placed in separate cages and given 20 Gm. each of unconditioned phenothiazine in feed. Ascaris, Oesophagostomum species, *Ascarops strongylina*, and *Hyostrongylus rubidus* were recovered from the feces. Weekly fecal examinations over a period of two months indicated that the animals were comparatively free of gastrointestinal parasites. Beginning with the third month in confinement, when the animals were approaching the age of 6 months, urine samples were obtained directly from the hogs at weekly intervals and examined for Stephanurus eggs. No eggs were found in the urine of hog 1 during the experimental period. In the urine of hog 2, a few eggs of *S. dentatus* were found. The number increased considerably by the seventh month to an estimated number of 100 eggs per cubic centimeter of urine.

In hog 3, a few eggs appeared irregularly during the sixth month and then the specimens became negative. It was maintained as a control and received no phenothiazine after the initial dose administered when the hogs were procured. Hog 2, sixty-five days after the initial treatment, received five 10-Gm. doses in feed at 3-, 4- and 5-day intervals over a period of 16 days.

Hog I received three 20-Gm. doses of phenothiazine at 5-day intervals for a period of 10 days. Erythrocyte and leucocyte counts and hemoglobin determinations were made before and after treatments. In the case of hog 2, blood counts were made during the 16-day period in which phenothiazine treatments were given. We were unable to observe any significant differences in these determinations in the three hogs. Thirteen days before slaughter, the hogs were weighed. Hog 1 (female) weighed 121 lb.; hog 2 (female), 103 lb., and hog 3 (barrow), 127 lb. Hog 2, which had received five 10-Gm. doses of phenothiazine, weighed the least and also revealed at slaughter that the infection by kidney worms had progressed further than in hogs 1 and 3, but liver lesions were not evident. Hog 1, which had never shown a positive egg count, revealed the most extensive liver lesions on gross examination.

In connection with these tests with phenothiazine for the removal of *S. dentatus* from hogs, observations were made on the effect this chemical and its oxidation products, thionol and phenothiazone, have upon the viability of the eggs of the parasite.

Unsuccessful attempts by Ross and Kauzal[2] to culture the eggs of *S. dentatus* in various dilutions of pure urine and tap water were reported in their work on the life cycle of this parasite; hence, cultures of this nature were not employed in this study.

Cultures were made of Stephanurus eggs obtained directly from the urine of hog 2 during treatment and ten days after the treatment period.

In the tests here recorded the urine was poured off the eggs, which were then washed once with tap water. The various solutions in 10-cc. volumes, as described below, were then poured onto the eggs remaining in the Petri dishes. In all cases the depth of the liquid in the dishes approximated 3 to 4 mm.

Group I.—This group comprised seven liquid controls, each consisting of 10 cc. of tap water to which had been added a few drops of fecal decoction filtrate. The fecal decoction was made by boiling a few grams of feces in water and then filtering through No. 12 filter paper. Eggs were collected two to ten days after the administration of phenothiazine. Fifty to 60 eggs were started in the solutions, resulting in a 100 per cent hatch in six cases and a 90 per cent hatch in one case. In three cultures

[8] Harwood, P. D., Jerstad, A. C., and Swanson, L. E.: The efficiency of phenothiazine for the removal of ascarids and nodular worms from swine. Sup. J. Parasitol., xxiv (1938), pp. 16-17.

100 per cent of the larvae reached the infective stage; in one culture 90 per cent matured; in one, 80 per cent matured; and in two cultures, 50 per cent reached the infective stage.

Group II.—In the second group, sterile soil and fecal decoction made from the feces of hog 2, receiving the phenothiazine, and collected at intervals of three to six days after treatment comprised the culture. Five cultures were made, each receiving 50 to 60 eggs. One similar culture made from feces obtained from hog 3, which did not receive phenothiazine, served as a control. After six days of incubation at room temperature the material in all cultures was transferred to Baermann funnels containing 85 per cent saline at 40 C. and allowed to stand for 24 hours. No larvae were recovered from the cultures made from material obtained from the hog receiving phenothiazine. Fifty-three infective larvae were recovered from the culture made from material obtained from hog 3, which had not been treated with phenothiazine.

Group III.—In group III, two sterile soil cultures, containing some fecal material to which was added just enough tap water to render the material semisolid, were employed. One one-hundredth of a gram of phenothiazine was sprinkled over the surface. Each of these cultures contained 50 eggs and both were obtained ten days after hog 2 received the last phenothiazine treatment. After six days the material was placed in Baermann funnels and drawn down at the end of 24 hours. No larvae were recovered.

Group IV.—In this group 10-cc. amounts of tap water containing fecal decoction were employed to which were added varying amounts of unconditioned phenothiazine, thionol, crude phenothiazone and urine product* from hog 1. Fifty to 60 eggs, obtained 10 days after the last treatment of the host animal, were distributed to each of the solutions. In concentrations of phenothiazine less than 0.005 per cent, eggs did not hatch. In concentrations of 0.1 per cent urine product from hog 1, eggs did not hatch. In concentrations of saturated solution of phenothiazone and thionol, respectively, diluted with equal volumes of water, the highest percentages of eggs hatching were 80 and 50, respectively. No larvae developed beyond the second stage. All were dead within three days.

Group V.—The uterus of one adult *S. dentatus* was placed in 0.85 per cent saline solution, severed, and the eggs allowed to escape into the medium. At the end of 24 hours at room temperature, 30 eggs containing live larvae were distributed to each of nine Petri dishes containing 10 cc. of tap water and a few drops of fecal decoction. To four of these solutions was added 0.01 Gm. of unconditioned phenothiazine. To one was added 0.005 Gm. of phenothiazine. Two dishes received 2 cc. and 1 cc., respectively, of a saturated solution of phenothiazone, one received 1 cc. of a saturated solution of thionol, and the control consisted of 10 cc. of tap water and a few drops of fecal decoction. In the thionol solution 83 per cent of the eggs hatched and the larvae developed to the infective stage. In the control 40 per cent of the eggs hatched and developed to the infective stage. In the solutions containing eggs where phenothiazine had been employed, with the exception of one culture, a few eggs hatched but did not reach maturity. No larvae reached maturity in any of the phenothiazone solutions. A few eggs, however, hatched in these cultures.

Group VI.—Live Stephanurus adults were incubated overnight at 37.5 C., and several hundred eggs thus obtained were placed in 10 cc. of sterile physiologic salt solution saturated with phenothiazine according to the method described by DeEds.[6] To each Petri dish was added one-half teaspoonful of fine sterile soil and fecal material combined. One control culture consisted of the same materials described above with the exception that it contained no phenothiazine. All cultures were 6 days old when they were placed in Baermann funnels. The control culture was the only one yielding live infective larvae.

DISCUSSION

In the field experiments wherein a modification of the BAI system of management for the prevention of kidney-worm infestation was tried, it appeared that such modifications in the system were sufficient to make the plan ineffective in preventing infestation with these parasites. It is considered doubtful, therefore, if any system of management less intricate and more practicable than the BAI plan based upon work in Georgia would be effective in controlling these parasites.

The failure of daily small quantities of phenothiazine to rid pigs of kidney worms may be explained in several ways:

1) The amounts given may have been

*One thousand cc. of urine was obtained from hog 1, which had been treated with 20 Gm. of phenothiazine. The urine was allowed to stand for a week; during this time the highly colored portion which periodically came to the top of the liquid was poured off and filtered through No. 12 fluted filter. These portions were combined and evaporated to dryness over a water bath. The product was redissolved in 95 per cent alcohol, filtered through No. 1 filter paper and allowed to evaporate to dryness at room temperature. One-tenth Gm. of the reddish brown product was placed in 100 cc. of distilled water. This solution was filtered through No. 12 filter and the filtrate in 10-cc. portions was employed; this is referred to as urine product.

insufficient; larger amounts may be effective.

2) Very little if any phenothiazine or its effective oxidation products are absorbed by the blood and reach the larvae by way of the blood stream.

3) Since the experimental pigs were 3 months old before treatment was begun, it may be that treatment, if started earlier, would have been more effective in destroying larvae while still in the liver.

The *in vitro* experiments dealing with different cultural methods on the viability of the eggs show that treating the feces of hogs with phenothiazine prevents the natural development of *S. dentatus* eggs and prohibits the larvae from reaching the infective stage. They also reveal that certain oxidation products of phenothiazine known to be excreted in the urine of treated animals, *e.g.*, phenothiazone and thionol, also have an inhibitory effect upon the development of the larvae.

These experiments, therefore, indicate that there may be at least two ways in which these results may have practical value:

1) Small quantities of phenothiazine sprinkled about the feeding troughs, houses and places where the sows most frequently urinate may prevent the eggs excreted in the urine from becoming infective for the pigs.

2) Repeated doses of phenothiazine administered to the sow during the period the pigs are with the sows may result in sufficient amounts of the oxidation products in the urine to prevent the eggs from becoming infective for the pigs.

It is felt that these results from *in vitro* experiments are sufficiently encouraging to warrant field experiments to determine their practical value.

SUMMARY

The results obtained by raising pigs according to the modifications of the U. S. Bureau of Animal Industry plan for preventing infestation by swine-kidney worms, as described in these experiments, were not encouraging.

Field treatments of hogs with small daily quantities of phenothiazine for the removal of the kidney worm, *Stephanurus dentatus*, concurrent with the described phenothiazine treatments of two hogs under controlled conditions, were not effective.

Stephanurus eggs obtained from hogs while under treatment with phenothiazine when washed in tap water and placed in tap water and fecal decoction cultures hatch and develop normally into infective larvae.

Attempts to rear infective larvae from eggs obtained from hog 2, in sterile fecal cultures made with feces from hog 2, during the same period as the eggs described in group 1 were unsuccessful, while eggs derived from the same source produced infective larvae in a sterile culture made from the feces of hog 3, which was receiving no treatment.

Eggs obtained from hogs ten days after treatment with phenothiazine when placed in sterile soil and fecal cultures prepared in Petri dishes and treated with 0.01 Gm. of phenothiazine, yielded no larvae.

Eggs obtained from hog 2 ten days after treatment with phenothiazine and eggs from adult worms obtained from untreated hogs when placed in saturated tap water solutions and 0.85 per cent saline solutions of phenothiazine, phenothiazone and thionol, to which were added a few drops of fecal decoction, or when diluted to one-half strengths, yielded no infective larvae. In one case, 1 cc. of a saturated solution of phenothiazone added to 10 cc. of tap water and fecal decoction inhibited normal development of larvae, although it did not prevent hatching of some of the eggs.

Eggs placed in 0.1 per cent urine product solution failed to hatch.

These preliminary experiments dealing with the inhibitory effect of the feces of hogs during treatment with phenothiazine upon the hatching of eggs and the development of larvae and similarly the effect of phenothiazine and its oxidation products, phenothiazone and thionol, are sufficiently encouraging to warrant further work along the line herein reported and also practical field tests with phenothiazine as a control agent against the infective larvae of *S. dentatus*.

An Experiment to Determine the Effect of the Growth Hormone of the Anterior Lobe of the Pituitary Gland on Swine*

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ANY MENTION OF past works on the pituitary gland that did not give prominence to the findings of Harvey Cushing's (1909-1910) early work would be slighting the real impetus that started the great mass of investigation that has gone on unceasingly since this century began. He removed the entire pituitary gland from dogs, and came to the conclusion that death always followed hypophysectomy within two or three days.

REVIEW OF LITERATURE

In 1912, Aschner used two puppies to check the findings of Cushing. His results did not agree with Cushing's. He reported that the hypophysectomized pup survived, but was stunted in growth.

Bell (1917), Benedict, Putman and Teel (1930), Dott (1923), Smith (1924-27), Danda and Reichert (1925), Reichert (1928) and many others agreed that the pituitary is essential for normal growth rate, and that if it is removed there immediately follows a cessation of growth.

This era of research was then forsaken for methods that would throw some light on the results of hyperactivity of the pituitary. The early workers in this phase took up where their predecessor left off, and after removing the pituitary, attempted to overcome hypofunction thus produced by feeding fresh and desiccated pituitary glands. Although an occasional investigator reported positive results from that procedure, there finally culminated the general belief that when the pituitary gland was given by the mouth, increased growth was not obtained. This positive fact was based upon the carefully controlled researches of Aldrich (1912), Hoskins (1916), Smith (1923) and Robertson (1916).

Allen (1916), after observing that Aldrich did not maintain normal growth in hypophysectomized toads by the feeding method, transplanted pituitary in normal toads and secured an increased growth. He also obtained slow growth by removing the pituitary from tadpoles. Crowe, Cushing and Holmans (1909) were able to prolong life in hypophysectomized animals by the use of transplants.

Transplants brought mostly disappointment and no real impetus was given the investigation of the growth hormone until Evans (1921-22) and coworkers showed that normal white rats could be grown into actual giants by intraperitoneal injections of an alkaline extract of the anterior lobe of the pituitary. These workers also found that female rats responded more uniformly to injections of growth hormone than did males. They found that growth response was best in unsexed injected females and least in normal injected males. Smith (1922-24-26-30) found almost complete growth stasis when he removed the pituitary from rats, but when daily injections of fresh rat pituitary were given, nearly normal growth was resumed. Van Dyke *et al.* (1930) and van Wagenen (1928) both produced increased growth by injections of the alkaline extract. Evans' original work with rats has been duplicated and verified on numerous occasions.

Watts (1935) approached the problem of the possible effect of growth-hormone injections by injecting mother rats and observing the rate of growth on their offspring. His research prompted him to report that the offspring from repeatedly injected females did grow more rapidly than the offspring of untreated female rats.

Putman, Benedict and Teel (1929) obtained excess growth in bull dogs, but a growth that was disproportionate, resembling acromegaly of man. Reichert (1928), after hypophysectomizing two Fox Terrier pups, was able to promote nearly normal growth by injections of a growth-hormone preparation. Evans *et al.* (1933) were able to show accelerated growth in dogs following injections. Handelsman and Gordon (1930) found evidence that the growth hormone accelerated periosteal growth of the bone.

Numerous investigators have shown that pituitary extracts influence certain metabolic processes. After experimenting with rats, Nilson, Palmer and Kennedy (1935) concluded that pituitary growth hormone promoted growth and increased the efficiency of dry-matter utilization and that female rats gave better growth response and dry-matter utilization than did males. Bierring and Nielson (1932) found increased nitrogen retention in rats following injections and Goebler (1933) found that following a single large injection of growth hormone dogs had a decided increase in nitrogen

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retention. Perla and Sandberg (1936) made a study of the effect of complete and partial hypophysectomy in adult rats on nitrogen, calcium and phosphorus retention. They found that the operated animals retained only about one half as much nitrogen as they did before having the pituitary removed. Calcium retention was less (it was excreted up to 600 per cent faster following hypophysectomy). Before the operation the rats retained 29 per cent of the phosphorus intake as compared with 6 per cent after the pituitary was removed.

Lee and Schaffer (1934) analyzed the carcasses of control and experimental rats and found that the untreated ones showed these characteristic changes with increased age: less water, less nitrogen, less ash and more fat. The carcasses of the injected animals, which were oversized, showed the same percentage composition in water, nitrogen, ash and fat as similar younger animals at the beginning of the experiment. Thus, he pointed out that the excess growth corresponded to that of younger animals and that the injected animals stored more nitrogen, ash and water, and less fat than the controls. The total storage of ash, nitrogen and water was greater, but because of the excess increase in growth the percentage of these elements was low in proportion to the age of the animal.

Handelsman and Gordon (1930) employed a unique method of studying the bone changes in rats injected with anterior pituitary extract. They found that by feeding madder (ground root of the plant *Rubric tinctorum*), which contains appreciable amounts of alizarin and purpurin, until the urine turned pink, newly growing bone stained a deep pink while old bone did not stain. These workers used rats that were about 100 days old and reported positive evidence of an overstimulation of normal periosteal growth after two weeks of injections.

Silberberg (1935-36-37) found that daily intraperitoneal injections of anterior pituitary extract in guinea pigs for three months caused enlargement of chondro-osseous junctions of the ribs and other purported changes. Samuels, Schott and Ball (1937) found that the removal of the pituitary of male rats caused them to have a lower sugar tolerance—even to the extent that they were completely diabetic five weeks after hypophysectomy. Sachs and McDonald (1925) reported an increased glucose tolerance in hypophysectomized dogs. Corkill, Marks and White (1934) obtained a similar result in rabbits. Teel *et al.* (1929-30) caused a decrease in urinary nitrogen by injecting pituitary-growth hormone into dogs.

Wilkins, Calhoun, Pilcher and Regen (1935) studied the influence of pituitary-growth hormone on phosphatase activities of the bones and kidneys. They used rats and showed that the injected animals had a lower phosphatase activity per unit of weight than did the controls.

Wallace (1935) reported normal serum-calcium values for hypophysectomized rats, but Riddle *et al.* (1936) found a decrease in the amount of serum calcium in several hypophysectomized pigeons. Houssay (1936) found that daily injections of alkaline extracts of anterior pituitary lobe resulted in a significant increase in the serum-calcium level of rats and guinea pigs. Later Friedgood and McLean (1937) reported an increase in serum calcium, but no significant change in serum phosphorus, following injections for nine days. Hogben and Charles (1932) and Dixon (1935) reported a decrease in serum calcium following injections of the pituitary extract.

After two years of research with hypophysectomized dogs, Benedict and Holmans (1912) found that:

1) Growth was retarded.
2) The gain in weight was due to an increased deposit of fat.
3) There was a lower body temperature.
4) There was a marked fall in pulse rate.
5) There was a decrease in respirations.
6) The total metabolic rate was decreased.

The literature covering the use of anterior growth hormone in dwarf children is abundant and is equally as conflicting.

Beck and Suter (1938) and Shelton (1937) report accelerated growth following treatment with the growth hormone. Struck and Szurek (1937) gave growth hormone to a dwarf and made a study of the nitrogen, calcium and phosphorus balance before, during and after treatment. There was an increased nitrogen retention, but calcium and phosphorus balances remained essentially unchanged during and after administration of the hormone. They reported that an increase in weight was the only change that was noticed in the patient.

Several authorities in the field of endocrine research doubt even the existence of a specific growth hormone. Riddle *et al.* (1933-36) stated that certain pituitary extracts owed their activity to a "balanced combination" of prolactin, thyrotropic and perhaps adrenotropic hormones. Their contention is based chiefly on the fact that while testing the crop response of pigeons to extracts high in prolactin, they secured an accelerated growth of the pigeons similar to that which had been attributed to the action of the growth hormone in other species. Evans, Simpson and Pencharz (1939) have attempted to answer Riddle's contention with recent investigations and, as a result of this piece of research, report that growth promotion in excess of normal secured by anterior pituitary extract is not dependent on the presence of the thyroid, but that growth is greater when the thyroid is present.

Freud *et al.* (1939) and coworkers fraction-

ated an alkaline growth-hormone preparation forty to one hundred times and secured a preparation that was "practically free" of prolactin, cortico and thyrotropic activity. These workers concluded that the excessive fractionation disposed of any possible balance of the hormones. Their research showed that growth ceased following hypophysectomy and that the epiphyses closed soon after the operation. They found that once the closure took place, it could not be reopened by treatment with growth hormone, but that if growth-hormone treatment was begun immediately after hypophysectomy, epiphyseal closure was prevented. They also concluded that the growth effect is localized in the epiphyseal cartilage, and that the growth process is analogous to that found in normal, young, growing animals.

Even though the latter experiments do not conclusively refute Riddle and coworkers' contentions, the importance of the thyroid and other hormones for growth in dwarf mice or pigeons does not necessarily disprove the existence of a specific growth hormone. Korenshevsky (1930) and Loeb *et al.* (1930) observed that following repeated injections of pituitary extracts, the animals so treated responded less and less to the administration of desiccated thyroid or thyroxin.

Selye, Collip and Thomson (1934) and Collip (1935) showed that rats develop an immunity to gonadotropic effects of implants of rat pituitary glands. Marrian and Butler (1937) showed that an immune serum may be developed in sheep by injections of sheep-pituitary gonadotropic preparations. Werner (1936), however, contended that the anti-hormones were formed in response to the administration of a foreign protein. In supporting his view he stated that the power of the beef-pituitary thyrotropic extracts to induce anti-hormone formation in the guinea pig depended upon the method of preparation, and Twombly (1936) stated that inactivated human pregnancy urine gonadotropic preparations were as effective in inducing anti-hormone formations as active preparations.

Object of the Experiment

Published experimental work seems to prove that the growth hormone of the anterior lobe of the pituitary gland will stimulate growth when injected into muscles or when given in the peritoneal cavity of white rats. The results of feeding the gland, although conflicting, indicate that this method is of doubtful value. Some experimental work has been reported dealing with dogs. The results obtained on human beings are debatable. It is believed that there is need for evidence on the effect the growth hormone will have on farm animals and man. This experiment was designed to try to answer the following questions:

1) Will the pituitary growth hormone produce increased growth in swine?

2) Will the sex or the sex condition of the animal influence the activity of the hormone?

3) Is the increased growth obtained by the treatments of normal composition?

4) Does the hormone affect the metabolism of calcium and phosphorus?

5) Are other glands of the body affected by injections of the growth hormone?

6) What histologic changes occur in the bones of the hormone-injected animals?

Animals Used and Plan of the Experiment

Eighteen purebred Hampshire, Berkshire and Tamworth pigs were selected and divided into six outcome groups, namely: A, B, C, D, E and F. Each outcome group was composed of litter mates of the same sex and sex condition. The outcome groups were divided into experimental group A, experimental group B, and control group. Wilson and Company's growth hormone preparation, phyone, was used in this experiment. The experimental group A received twice a week 3 cc. of phyone per 100 lb. of live weight injected intraperitoneally, and experimental group B received 6 cc. per 100 lb. of live weight. The control group received no growth hormone.

TABLE I—Arrangement of Outcome Groups

Outcome Groups	Experimental Group A	Control Group	Experimental Group B
A (barrows)	Pig 110	Pig 111	Pig 112
B (barrows)	113	114	115
C (barrows)	116	117	118
D (gilts)	119	121	122
E (gilts)		123	125
F (gilts)		126	127

Outcome group A was composed of Berkshire barrows; outcome group B, Tamworth barrows; outcome group C, Hampshire barrows; outcome groups D and F, normal Hampshire gilts; and outcome group E, normal Berkshire gilts. This experiment was started on February 24, 1938. All of the pigs were farrowed in the fall of 1937 and ranged in weight from 110 to 127 lb.

They were fed a mixture of 90 lb. of yellow corn, 5 lb. of 60 per cent tankage, 5 lb. of 43 per cent protein cottonseed meal and 3 lb. of steamed bonemeal. Each pig received one-half ounce of cod liver oil three times a week.

Each pig was placed in an individual pen

with a small outside runway and fed from a self-feeder. The feed was weighed into the feeder once a week and the portion that remained at the end of the week was subtracted in order to determine the amount eaten.

Each animal was weighed on three consecutive days at the beginning and at the end of the experiment. Biweekly weights were taken and the following measurements were made each time:

1) Length of back.
2) Circumference of heart girth.
3) Length of tibia, metatarsus and radius.

The percentage of serum calcium was determined by the method of Roe and Kahn.[1] The percentage of serum inorganic phosphorus was determined by the Fiske and Subbarow[2] method. The percentage of hemoglobin also was determined every two weeks, by the Newcomer method.[4, 5]

After 14 weeks the pigs were slaughtered and the following information obtained:

1) Dressing percentage.
2) Percentage of total nitrogen in the latissimus dorsi muscle.
3) Percentage of water in the latissimus dorsi muscle.
4) Weight of kidneys, liver, uterus, lungs, heart, spleen, ovaries, adrenals and pituitary gland.
5) Percentage of total ash in fibula.
6) Length of tibia, metatarsus and radius.
7) Histologic studies of the middle of the proximal end of the tibia.

RESULTS OF THE EXPERIMENT WITH SWINE

Although 18 pigs were started on the experiment, two were eliminated on March 10 due to necrotic enteritis. The three Berkshire barrows—110, 111, 112—also developed the disease, but soon overcame the acute symptoms. They remained unthrifty, however, until about the middle of the experiment.

Total Gain in Weight.—The pigs which were injected with 3 cc. of phyone per 100 lb. of weight had a mean gain of 155 lb. The paired controls had a mean gain of 144 lb. The mean difference 11.±15. is not significant according to Fisher's T test. Only outcome groups A, B, C and D were used because two pigs in the light injected group and in outcome groups E and F were removed early in the experiment and thus eliminated the paired condition that was established in those outcome groups.

All six outcome groups were tested in the comparison between the control group and the group that received 6 cc. of phyone per 100 lb. The injected group had a mean gain of 146 lb. and the control group had a mean gain of 140 lb. The difference 6.±11. is not significant. Although there was no real difference, four of the injected animals gained more than their controls.

Measurements.—Although measurements were made according to the plan of the experiment, it was soon evident that these data would not be of any value in drawing a conclusion as to the effect of the growth hormone. The length of the back from the base of the skull to the base of the tail proved more accurate than the other measurements.

When the controls were compared with the heavily injected group, six pairs were tested. The controls had a mean gain in back length of 8.5 in. and the experimental group had a mean gain in length of back of 9.75 in. Although a trend is indicated, the difference is not real. The mean difference is 1.25±.66.

Percentage of Serum Calcium and Serum Inorganic Phosphorus.—Nine determinations were made at intervals of approximately two weeks to determine the percentage of serum inorganic phophorus and serum calcium present. The data thus collected failed to show any consistent difference in the amount of the serum calcium or serum inorganic phosphorus in the blood of the injected groups and the control groups. The injected groups had a mean serum calcium of 10.5 mg. and 7.6 mg. of serum inorganic phosphorus per 100 cc. The control groups had a mean serum calcium of 10.5 mg. and a mean of 7.5 mg. of serum inorganic phosphorus per 100 cc. These are normal values for pigs of this age.

Hemoglobin. — Hemoglobin determinations were made on nine occasions during the course of the experiment. Although the results on each individual pig varied

[1]Roe, J. H., and Kahn, B. S.: Colorimetric determination of blood calcium. J. Biol. Chem., lxxxi (1929), pp. 1-8.

[2]Fiske, C. H., and Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem., lxvi (1926), pp. 375-409.

[4]Newcomer, H. S.: Absorption spectra of acid hematin, oxyhemoglobin and carbon monoxide hemoglobin. J. Biol. Chem., xxxvii (1919), pp. 465-496.

[5]*Ibid.*: A new optical instrument for the determination of hemoglobin. J. Biol. Chem., lv (1923), pp. 569-574.

slightly from one test to another, the trend was never consistently toward an increase or decrease. There was no difference in the amount of hemoglobin in the blood of the control animals and the injected animals. The mean for the injected group was 11.44 Gm. per 100 cc. of blood and the mean for the control group was 11.24 Gm.

Postmortem Examination.—Experimental groups A and B failed to show any real difference in dressing percentage from their controls. Group A had a mean dressing percentage of 79.9 and the controls, 79.6. The mean difference 0.3 ± 1.16 is not real.

The six animals which received 6 cc. of phyone per 100 lb. of weight had a mean dressing percentage of 77.0 and the controls, 78.4. The mean difference $0.4 \pm .90$ is not significant.

The small intestines were measured from each animal at slaughter and the length determined. The shortest, 58 ft., was found in pig 116, and the longest, 68.5 ft., was found in pig 119. Experimental group A had a mean intestinal length of 64.6 ft.; experimental group B, 64 ft.; and the control group, 65.2 ft. The mean length for the 16 pigs was 64.6 ft. There was no evidence that the growth hormone determined the length of the small intestines.

A comparison of the gain in size of the heart girth between the light injected group and the controls showed no real difference. The injected group had a mean gain of 11.75 in. and the control group, 11.5 in. The mean difference $.25 \pm .44$ was not due to the growth-hormone treatment.

The heavier dosage of phyone did not cause the experimental group B to have a larger heart girth than the controls. The mean change of the injected group was 9.83 in. and of the control group, 8.5 in. The mean difference was 1.33 ± 1.10.

The length of the femur, tibia, fibula, humerus, radius and ulna was measured. There was no significant difference found between the injected animals and the control animals. In fact, the only value derived from these measurements was to show the inaccuracy of the measurements taken before the animals were slaughtered.

Each liver was weighed at slaughter. In three of the litter-mate groups the liver was heavier in the injected animals than in the control animals, but in the three remaining groups each of the controls had a larger liver than either of the injected animals in the group. The weight of the kidneys, heart, lungs, spleen, ovaries, adrenals and the pituitary body was taken, but showed no indication that these organs had been influenced by the repeated injections of the growth hormone.

Chemical Analyses.—Moisture Content of Muscle: A comparable sample was taken from the latissimus dorsi muscle of each pig. All external fat was removed by scraping the muscles as clean as possible. Each sample was ground and mixed three times in order to secure a sample. Samples of approximately 2 Gm. were taken and dried at 100 C. for 24 hours. Triplicate samples were run on each.

The four animals which received 3 cc. of growth-hormone preparation were compared with their paired controls and each group had nearly identical means. The mean moisture content of the injected group was 71.6 per cent and the control group, 71.9 per cent. The mean difference $0.3 \pm .12$ is not real.

The mean difference and the standard error of the mean difference also were determined on the heavy injected group and the control group and tested for significants. The mean of the control group was 72.6 per cent moisture as compared with 72.2 for the injected group. The mean difference of 0.4 ± 0.40 is not the result of the repeated injections of the hormone.

Protein Determination of Muscle: A 2-Gm. sample of the ground latissimus dorsi muscle was used to determine the percentage of nitrogen in muscle. A macro-Kjeldahl was run in triplicate and the nitrogen thus determined was considered to be 16 per cent of the total protein of the muscle. The light injected group had a mean of 20.4 per cent and the control group had a mean of 20.3 per cent. The mean difference $0.1 \pm .52$ is due to chance. The difference of $0.6 \pm .54$ found between the heavy injected group and the control group is too small to be attributed to the action of the growth hormone. The mean of the controls was 20.1 per cent and the injected

animals had a mean of 20.7 per cent protein in their muscles.

Total Ash Determination of the Fibula: The left fibula was removed from each pig, crushed into small pieces and all of the fat extracted. Each bone was then dried to a constant weight and ashed at 538 C. for one hour.

Pigs 110, 111 and 112, which were unthrifty following an attack of necrotic enteritis during the experiment, showed a total ash percentage of 54.2, 55.3 and 56.6 respectively. All of the other pigs in the experiment ranged from 61.6 to 66.6 per cent. With all of the pigs included in the calculation, the injected pigs had a fibula ash content of 62.5 per cent, and the controls, 61.6. If the three pigs—110, 111, 112—are eliminated from the calculations, the injected group had 64.7 per cent as compared with 63.2 per cent for the controls.

The light injected group and the control group had identical means of 61.6 per cent. The mean difference between the heavy injected animals and their controls was highly significant. The injected pigs had a mean of 63.2 per cent ash in the fibula and their controls had 61.7. The mean difference $1.58 \pm .30$ is real and is due to the influence of the growth-hormone treatments. Of the 14 bones ashed, 9 were from the injected pigs and 5 from the controls. Seven of the 9 injected animals showed a higher ash percentage than did their controls.

Materials and Methods Used in Bone Study

A section of bone for histologic examination was taken from the middle of the proximal end of the tibia of each pig. The section was so cut that it contained the epiphyseal cartilage, or plate, with the upper end of the diaphysis and the lower end of the epiphysis. The specimens were fixed, washed, hardened and decalcified.

Results of Histologic Study

Pig 110, a light injected animal, had an area of mature and new cartilage $1,073\mu$ wide, and pig 112, a heavy injected animal, 956μ as compared with 886μ for number 111, the control.

In the next group of pigs the light injected pig showed a cartilage area of 675μ, the heavy injected animal, 758μ, as compared with 245 in the control.

In the third group the light injected pig had a cartilage area of 538μ while the pig that received 6 cc. of phyone per 100 lb. of live weight twice weekly had an area of calcification $1,003\mu$ wide; that of the control was 653μ wide.

The light injected pig in the next group had a total width of new and old cartilage of $1,400\mu$, the heavy injected, 1,600, and the control, 960.

Group 5, which was composed of only two pigs, was the only group which failed to show increased cell proliferation in the injected pig. In this group the plate in the injected pig was but 525μ wide, while in the control animal it was $1,470\mu$.

The last group was also composed of only two pigs. The control animal's area of proliferating cartilage was $1,960\mu$ wide, and the injected animal, 2,150.

Discussion

One of the objectives of this experiment was to determine if repeated injections of the growth hormone of the anterior lobe of the pituitary gland would promote increased growth in swine. Growth is the increase in the size of the skeletal and muscle tissues. In view of this definition, the fact that there was no significant difference in the total number of pounds gained by the control group and the injected group does not mean that there was not a difference in actual growth. The mean $11. \pm 15.$ between the light injected pigs and the controls was not significant; neither was the mean difference of $6. \pm 11.$ between the control and heavy injected group real.

Evans and Long[3] failed to obtain a difference in the weight of their injected and control white rats until the rats had reached maturity. It is entirely possible that had the injections of growth hormone been continued until the pigs approached maturity, increased or continued growth would have been obtained.

Although this experiment did not make comparisons between altered and normal

[3]Evans, H. M., and Long, J. A.: Characteristic effects upon growth, oestrus, and ovulation induced by intraperitoneal administration of fresh anterior hypophyseal substance. Anat. Rec., xxiii (1922), p. 19.

animals of the same sex, it did give opportunity to observe the action of the growth hormone on normal female pigs and barrows. Evans and Long[3] secured the greatest growth response in normal female rats and the least growth response in uncastrated male rats. Their castrated male rats grew nearly as large as did the uncastrated female rats.

Measurements of the length of the back, tibia, metatarsus and radius proved to be of no value in determining the growth from week to week. It was impossible to measure between the same two points each time, and the error of measurement was further increased by the pig's not having its muscles in the same state of contraction at each measurement. These data proved of no value in interpreting the results of the aforementioned investigators' experiment. Neither macroscopic examinations nor chemical analyses revealed any increased growth in the muscles of the injected pigs.

Moisture determinations were made on a sample of the latissimus dorsi muscle from each pig. The pigs that were given 3 cc. of phyone per 100 lb. of weight had a mean moisture content of 71.6 per cent as compared with a mean of 71.9 for the control pigs. The mean difference of $0.3 \pm .12$ is not real.

A comparison of the heavy injected animals with their paired controls gave a mean difference of only $0.4 \pm .40$. The mean of the injected animals was 72.2, and the control pigs had a mean of 72.6. The sex of the animal did not affect the moisture content of the muscle, nor did repeated injections of growth hormone. This is not in accord with the findings of Lee and Schaffer.[6] They found that the muscles of injected rats were higher in percentage of water than the controls.

Protein determinations, which were made on the latissimus dorsi muscle of each pig, failed to show that the injections of growth hormone had caused any change in the amount of the protein in the muscle. The mean difference between the light injected group and the control group was only $0.1 \pm .52$. The mean difference between the control pigs and the heavy injected pigs was $0.6 \pm .54$.

These results are not in accord with those of Lee and Schaffer.[6] They reported that the carcasses of their injected rats had a higher content than did the controls. It is unusual that their work should have shown that the carcasses of the injected animals contained a higher percentage of water and protein, for this would have meant that the injected animals had less fat. Most investigations have shown that the increased weight of the injected rats was due to an excess fat accumulation. Bierring and Nielson,[7] Goebler[8] and Struck and Szurek[9] reported that there was an increased nitrogen retention in their injected subjects, but neither showed the muscles to be richer in nitrogen.

The injected animals had a mean of 10.5 per cent of serum calcium and a mean of 7.6 per cent of serum inorganic phosphorus, as compared with 10.5 per cent calcium and 7.5 per cent serum inorganic phosphorus for the controls. These figures are within the ranges given for normal animals. The findings of previous investigators are in conflict. Houssay,[10] working with rats and pigeons, reported an increase in the percentage of plasma calcium. Friedgood and McLean[11] reported that their injected animals showed an increase in serum calcium, but no change in the serum phosphorus. Earlier, Hogben and Charles[12] and

[6]Lee, M. O., and Schaffer, N. K.: Anterior pituitary growth hormone and the composition of growth. J. Nutri., vii (1934), pp. 337-363.

[7]Bierring, E., and Nielson, E.: The composition of the tissues of albino rats treated with alkaline pituitary extracts. Bio-Chem. J., xxvi (1932), pp. 1015-1021.

[8]Goebler, O. H.: Some effects of anterior pituitary extracts on nitrogen metabolism, water balance, and energy metabolism. J. Exp. Med., lvii (1933), pp. 349-363.

[9]Struck, H. C., and Szurek, S. A.: A study of a dwarf under treatment with growth hormone. Endocrinol., xxi (1937), pp. 387-393.

[10]Houssay, B. A.: Certain relations between the parathyroids, the hypophysis, and the pancreas. New Eng. J. Med., ccxiv (1936), pp. 1128-1136.

[11]Friedgood, H. B., and McLean, R.: The effect of anterior hypophyseal extract upon the serum calcium and phosphorus. Am. J. Physiol., cxviii (1937), pp. 588-593.

[12]Hogben, L., and Charles, E.: Studies on the pituitary. IX. Changes in blood calcium following injections of anterior lobe extracts and sexual excitement in female rabbits. J. Exp. Biol., ix (1932), pp. 139-148.

Dixon[13] reported a decrease in the percentage of calcium in the serum of their injected animals.

The primary source of blood calcium is the food, but its level is not readily influenced by the dietary intake. The level of the blood calcium is largely controlled by the parathyroid hormone. If the blood calcium becomes depleted, this hormone causes a rapid replacement of it from the bones. The parathyroid is under the influence or control of the pituitary gland. No evidence is available, however, that would indicate that the growth hormone is responsible for the activity of the parathyroid gland; neither is there any evidence that the growth hormone preparation, phyone, used in this experiment is contaminated with the hormone that regulates the secretion of the parathyroids. These facts seem to point out that a pathologic state would have to exist in the animal before significant changes in the serum calcium or serum inorganic phosphorus could exist.

Data have been reported which prove that the pituitary gland exerts a specific control over the secretory activity of most of the other glands of internal secretion. These data do not show that a hyperactivity of the pituitary gland necessarily causes a hypertrophy or an atrophy of those affected glands; neither does it follow, when the various glands have their rate of secretion changed, that they necessarily undergo a change in size.

Smith[14] did show that hypophyseal transplants prevented atrophy of the genitalia, which usually accompanied hypophysectomy. Shockaert,[15] Loeb[16] and Janssen and Loeser[17] also showed that repeated injections of extracts of the anterior lobe of the pituitary gland caused hypertrophy of the thyroid gland. Smith[18] reported that the adrenal cortex rapidly atrophied following hypophysectomy. All of these investigators, and many others, have shown the interrelationship of the pituitary gland with the other glands of internal secretion. No work has been presented which shows the growth hormone to be responsible for those conditions.

The liver, kidneys, heart, lungs, spleen, ovaries, adrenals and pituitary gland were weighed and showed no indication that they had been influenced by the repeated injections of the growth hormone.

Repeated injections of growth hormone stimulated the growth of bone in swine. Histologic studies revealed a wider area of proliferating cartilage in the tibia of the injected pigs than in the litter-mate controls. This wider area of cartilage was not due to impaired calcification, but to the presence of a wider area of new, rapidly growing cartilage. If rickets had been present in these pigs, either a lowered serum calcium or a lowered serum inorganic phosphorus would have been encountered. By the ash determinations which were made, it was further shown that rickets was not involved.

The measurable data which were collected in the bone studies are given in table II.

Every injected pig, with the exception of No. 123 (table II), in each outcome group showed a wider area of cartilage proliferation than the control in its respective group. It is possible, but not probable, that the tibia specimens of pigs 123 and 125 became mixed. Those data are presented as collected and the measurements of the tibia specimens from pigs 123 and 125 show the tibia of pig 123 to be growing faster than that from pig 125.

The percentage of ash was higher in the fibula of each injected pig than was found in its control, with the exception of barrows 110 and 113.

Roentgenographic studies of the left

[13]Dixon, T. F.: The influence of ovarian and anterior pituitary hormones on calcium metabolism. Bio-Chem. J., xxvii (1933), pp. 410-418.

[14]Smith, P. E.: Ablation and transplantation of the hypophysis in the rat. Anat. Rec., xxxii (1926), p. 221.

[15]Shockaert, J. A.: Enlargement and hyperplasia of the thyroids in the young duck from the injection of anterior pituitary. Am. J. Anat., xlix (1932), pp. 379-408.

[16]Loeb, L., *et al.*: Effect of hormone of anterior pituitary on thyroid gland in guinea pigs. Proc. Soc. Exp. Biol., New York, xxvi (1929), pp. 860-862; xxvii (1930), pp. 490-492; xxviii (1930), pp. 209-213.

[17]Janssen, S., and Loeser, A.: Del Wirkung des Hypophysenvorderlappens auf die Scheldruse. Arch. exp. Path. Pharmak., clxiii (1931), pp. 517-529.

[18]Smith, P. E.: Hypophysectomy and a replacement therapy in the rat. Am. J. Anat., xlv (1930), pp. 205-273.

TABLE II—Data Collected in Bone Studies

Pig No.	Width (in Microns) of Proliferating Cartilage of Tibia	Percentage of Ash in the Fat and Moisture-Free Fibula
110	1,073	54.2
111*	886	55.3
112	956	56.5
113	675	64.0
114*	245	65.7
115	758	66.6
116	538	64.6
117*	653	63.8
118	1,003	65.2
119	1,400	63.7
121*	960	62.0
122	1,600	64.5
123*	860	61.6
125	310	63.5
126*	1,960	Lost in ashing
127	2,150	Lost in ashing

*Control.

fibula of each pig substantiated the facts obtained in the histologic and ash studies of the bone. Each outcome group showed a greater degree of calcification in the injected animals than in the control, with the exception of group E. In that group the fibula from the control gilt 123 showed more calcification than the injected pig 125.

A hormone usually exerts a more noticeable influence when injected into animals that have deficiency of that particular hormone. This experiment was conducted on animals which had no hyposecretion from the pituitary gland. More striking growth response would probably have been secured if the injections had been made into hypophysectomized pigs.

Summary and Conclusions

Ten pigs approximately 20 weeks of age and weighing from 110 to 137 lb. were given intraperitoneal injections of the growth hormone of the anterior pituitary gland twice weekly for 4½ months. Four of the pigs received biweekly doses of 3 cc. of growth hormone per 100 lb. of live weight, and six received 6 cc. per 100 lb. of live weight. Each of these pigs was compared with a litter-mate control of the same sex and sex condition. The data collected from this experiment seem to warrant the following conclusions:

1) Biweekly doses of 6 cc. of phyone given intraperitoneally per 100 lb. of weight to pigs do not produce noticeable increased growth within 4½ months of treatment.

2) Pigs so treated were not more efficient in the use of their feed than untreated animals, and did not dress any higher or lower percentage than the untreated animals.

3) The length of the small intestines was not affected by the repeated injections of growth hormone.

4) The weight of the kidneys, liver, uterus, lungs, heart, spleen, ovaries, adrenals and pituitary gland was not influenced by the hormone treatment.

5) The percentage of moisture and nitrogen in the muscle was not affected by the hormone treatment.

6) Neither the grams of hemoglobin nor the percentage of serum calcium and serum inorganic phosphorus were affected by repeated injections of the hormone.

7) Repeated injections of the hormone produced an increased calcification in the fibula as was shown by roentgenographic studies.

8) The total ash percentage of the fibula was greater in the injected group. Seven of the nine hormone-treated pigs had a higher ash content than did their controls. The dose of 3 cc. did not cause a significant increase, but the 6-cc. dose caused a highly significant increase.

9) The growth hormone of the anterior lobe of the pituitary body appears to stimulate growth at a definite anatomic point. A microscopic study of sections made from control and hormone-treated pigs showed an increased proliferation of the hyaline cartilage separating the bone structure of the diaphysis and epihyses in the hormone-treated pigs. The hormone seems to be strictly chondrotropic in its activity in swine.

10) Barrows and gilts responded alike to repeated injections of the hormone.

Further Studies on Overeating (Enterotoxemia) of Feedlot Lambs*

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A CONDITION DESIGNATED as overeating is responsible for many losses in feedlot lambs during that period of the fattening process when a heavy grain ration is fed. The deaths are usually among the largest and thriftiest lambs. It has been observed for a number of years that the losses can be reduced by decreasing the grain ration one half to three fourths of the amount being fed when the deaths occurred.

Except that sudden deaths may occur when lambs are fed a heavy ration, the etiology and pathogenesis of overeating are not well understood. It was determined in previous work[1] that the antiserums for the toxins of *Clostridium welchii* types B and D will neutralize the toxin found in the intestinal tract of many of these cases. This was not true of the antiserums of types A and C of the *Cl. welchii* toxin.

It is assumed, as a result of these findings, that overeating is due to the multiplication of *Cl. welchii* type D and the production of its toxin in the intestinal tract, and that the consumption of a large amount of concentrate feed produces the conditions favorable for the growth of that organism.

This paper is a report of further studies on this suspected etiologic agent as it relates to overeating in lambs.

Toxicity of Intestinal Filtrates

In previous reports from this station[1, 2] it was shown that filtrates prepared from the contents of the intestinal tracts of lambs dead of overeating were frequently toxic for laboratory animals. In one series of 256 overeating cases, 45 per cent of the filtrates were toxic, while only 2 out of 87 filtrates prepared from lambs dead of other causes were toxic for laboratory animals. In another series, 83 per cent of the filtrates from 54 cases diagnosed as overeating were toxic for rabbits and white rats. These results were in agreement with those of Bennetts,[3] who reported the presence of a toxin in the intestinal tract of lambs which had shown symptoms and lesions similar to those observed in the overeating cases of this area.

In this study, including the 1940-41 season, filtrates were again prepared from the intestinal contents of 41 overeating cases. When intravenously injected into rabbits, 75.6 per cent of these were toxic. Of 30 cases with diagnoses other than overeating, two, or 6.6 per cent, showed the presence of a toxin in the intestinal tract. One was a week-old lamb suffering from pneumonia and enteritis. The other was a feeder lamb with many of the usual gross lesions of overeating, but with a well-developed pneumonia. The results of these tests are similar to those previously recorded.

We can safely assume, as a result of these tests, that a toxin is present in the intestinal tract of the greater number of lambs dead of overeating and that the toxin is seldom present in lambs dying from other causes. Assuming further that the toxin is absorbed, the name enterotoxemia would be appropriate for the condition.

Activation of Toxin

In 1940, it was reported[4] that difficulty was encountered in getting a potent toxin in culture of strains of *Cl. welchii* isolated from lambs which died of overeating. Further trials were made with various meat broths to which meat particles had been

*Paper No. 127, scientific journal series, Colorado Experiment Station, Pathology and Bacteriology Section.

[1]Newsom, I. E., and Thorp, F. Jr.: The toxicity of intestinal filtrates from lambs dead of overeating. J.A.V.M.A., xciii (1938), pp. 165-167.

[2]Colorado Exp. Sta., 52nd Ann. Rpt. (1939).

[3]Bennetts, H. W.: Infectious enterotoxemia of sheep in western Australia. Australian Counc. for Sci. & Ind. Res., Bul. 57 (1932).

[4]Colorado Exp. Sta., 53rd Ann. Rpt. (1940).

added. Different periods of incubation were tried. Fresh horse meat or beef-heart broth and an incubation period of four days were found to give the most satisfactory results. A longer period of incubation favored a greater production of the "x" factor of the toxin. Not all of the experimental strains produced toxin and, therefore, after preliminary trials the work was confined to three cultures. The most satisfactory was a strain recently isolated.

At no time was a culture toxin produced which was as potent as the greater number of the intestinal filtrates prepared from overeating cases. When rabbits were used as the test animals, death was commonly produced in from three to six minutes with doses of 1 to 2 cc. of filtrate administered intravenously. With doses of 0.5 cc. and smaller, death could usually be delayed for a period of 15 minutes to several hours. Filtered cultures of *Cl. welchii* either were nontoxic in comparable doses or required an interval several times greater to kill.

Bosworth and Glover[5] discovered that the filtrates prepared from the intestinal contents of normal lambs contain a substance which activates the toxin of *Cl. welchii* type D. This substance was identified as the pancreatic enzyme, trypsin. They found that trypsin increased the toxicity of dried toxin 30 to 40 times its original value. A similar, but not as marked, effect was produced by the enzyme action in liquid toxin from culture.

Trials were made to determine the effect of pancreatin, pepsin and filtrates prepared from the contents of the abomasum and intestine of normal lambs on the Seitz-filtered broth of incubated cultures of *Cl. welchii* isolated from overeating cases. The enzymes were used either in powdered form or mixed with distilled water to make a weak solution. Rabbits were inoculated intravenously. The results of these trials are shown in table I.

From these experiments it may be seen that pancreatin and intestinal filtrates from normal lambs contain a substance which markedly increases the potency of culture toxin. Some cultures which were nontoxic in doses up to 1 cc. when given alone were found to be very toxic when either of these substances was added. Others which were slow in killing had their potency increased approximately six times by the addition of one of these substances, and the interval required to kill approached that for toxic intestinal filtrates from overeating cases. Like effects were not produced by filtrates prepared from normal abomasum contents and pepsin. Although the increase in potency in these trials is not as great as reported by Bosworth and Glover,[5] the difference may be due to the fact that dried toxin was used by them in most of their trials. In the tests in which they used liquid toxin, the increase in potency was not as great.

In another trial, 0.8 cc. of a 24-hour broth culture of *Cl. welchii* was inoculated into two tubes of Seitz-filtered intestinal contents from a normal lamb. One of the tubes had been heated at 70 C. for 20 minutes prior to inoculation. Incubation was at 37 C. for two days. Growth was very slow, for few organisms were found in smears. Five-tenths cc. of the unheated product injected intravenously in a rabbit produced symptoms in twelve minutes and death in four hours. The same dose of the material which had been heated prior to inoculation produced no symptoms.

EFFECT ON BLOOD SUGAR

In the series of autopsies of lambs which died in feedlots during the 1940-41 feeding season, glycosuria was found in 16, or 59 per cent, of 27 overeating cases from which urine was obtained. Four overeating cases were submitted from which it was possible to obtain blood samples for sugar determination before death. One of these was within the normal range with 66 mg. per 100 cc. The other three were 107, 140 and 220 mg., respectively.

During the toxicity tests of intestinal filtrates on rabbits, a filtrate was occasionally encountered which in small doses was only slowly toxic. As an example, an in-

[5]Bosworth, T. J., and Glover, R. E.: The effect on *Cl. welchii* toxins of a substance present in the normal intestine. Rpt. Inst. Animal Path., Univ. of Cambridge (1934-35), pp. 79-93.

TABLE I—Effect of Enzyme-Containing Substances on Culture Toxin of Cl. Welchii Isolated from Lambs

TOXIN	AMT. (CC.)	SUBSTANCE ADDED	AMT. (CC.)	pH	INTERVAL BEFORE INJECTION	RESULT
A	0.5	———	——	——	——	Nontoxic
A	0.5	Normal intestinal filtrate	0.5	——	3½ hrs. room temp.	Down 8 min.; dead 18 min.
B	0.5	———	——	——	——	Nontoxic
B	0.5	Normal intestinal filtrate	0.5	——	3½ hrs. room temp.	Nontoxic
C	1.0	———	——	——	——	Nontoxic
C	1.0	0.2% pancreatin	0.5	——	30 min. room temp.	Down 10 min.; dead 15 min.
D	1.0	———	——	——	——	Killed 1 hr., 45 min.
D	1.0	0.5% pancreatin	0.1	——	24 hrs. 37 C.	Down 2 min.; dead 16 min.
E	0.4	———	——	6.85	———	Nontoxic
E	0.4	Pepsin (pwd.)	——	4.85	2½ hrs. room temp.	Nontoxic
E	0.4	Normal intestinal filtrate	0.4	7.1	2½ hrs. room temp.	Killed 1 hr., 30 min.
E	0.4	Normal intestinal filtrate in media inoc. for toxin		6.9	———	Killed 2 hrs., 8 min.
F	0.2	———	——	6.9	——	Nontoxic
F	0.2	———	——	4.2	——	Nontoxic
F	0.2	Pepsin (pwd.)	——	4.2	1½ hrs. room temp.	Nontoxic
F	0.2	Normal abomasum filtrate	0.2	6.77	1¼ hrs. room temp.	Nontoxic
F	0.2	Normal intestinal filtrate	0.2	7.1	1¼ hrs. room temp.	Killed 4 hrs.
F	0.2	Normal cecum filtrate	0.2	6.95	1¼ hrs. room temp.	Killed 36 hrs.
F	0.2	Normal intestinal filtrate in media before inoc. for toxin	——	——	——	Sick 1½ hrs.; recovered
G	0.5	———	——	——	———	Killed 1 hr.
G	0.5	Normal intestinal filtrate in media before inoc. for toxin	——	——	———	Killed 10 min.
G	0.5	Pancreatin (pwd.)	——	——	1¼ hrs. room temp.	Killed 17 min.
G	0.5	Pepsin (pwd.)	——	——	1¼ hrs. room temp.	Killed 4 hrs., 23 min.
G	0.5	Pepsin (pwd.)	——	4.7	1 hr., room temp.	Killed 4 hrs.
Sterile broth	1.0	0.2% pancreatin	0.5	——	2 hrs., room temp.	Nontoxic
	——	Normal intestinal filtrate	1.0	——	———	Nontoxic

travenous injection of 0.2 cc. of Seitz filtrate 125 killed a small rabbit in six hours. The urine from the bladder of this rabbit was positive for sugar with Benedict's reagent.

To determine whether a glycemia is commonly present, to account for the glycosuria and to compare the effect of intestinal filtrates and toxin on the blood-sugar level, small doses of toxic intestinal filtrates, culture toxin and enzyme-activated toxin were injected intravenously into a series of rabbits and two lambs. In the rabbits, blood samples were taken from the heart. Ether anesthesia was employed, except for those samples taken just before death. The results of these trials are shown in table II.

It will be noted that with one exception (rabbit 3) there was a decided increase in blood sugar after the inoculation of rabbits with the toxic material, whether it was intestinal filtrate, culture toxin alone or activated toxin. Rises of from 106 mg. to 242 mg. over the pre-injection level were recorded. There were somewhat larger increases when toxin from culture and activated toxin were injected than when the intestinal filtrates were employed. Normal intestinal filtrate and sterile broth produced some increase, but the rise was not as great as when the toxic substances were used. Three of six urine samples taken from the bladder at autopsy contained sugar. Lamb B, which was killed with an intestinal filtrate, showed an elevation in blood sugar of 124 mg. over the pre-injection level, while in lamb 134, which showed no symptoms, only a slight rise was recorded.

REPRODUCTION OF THE OVEREATING SYNDROME

Rabbits and sheep may be readily killed by the intravenous injection of toxic intestinal filtrates or of the toxin produced in culture of *Cl. welchii* type D. The references to the reproduction of toxemias with *Cl. welchii* type D or its toxins given by the alimentary tract are few. Bennetts[6]

[6]Bennetts, H. W.: Infectious enterotoxemia, pulpy kidney, and other related diseases of sheep. Vet. J., lxxxviii (1932), pp. 248-256.

stated that enterotoxemia was successfully reproduced in six experimental animals by drenching with doses of 50 to 200 cc. of cultures of *B. ovitoxicus* (*Cl. welchii* type D) after intestinal sluggishness was induced with opium and belladonna. Oxer[7] and Gill[8] were unable to produce pulpy kidney disease by Bennetts' method. Roberts[9] succeeded in producing a disease in lambs which closely resembled the naturally occurring pulpy kidney disease by feeding toxin-free *Cl. welchii* type D bacteria in conjunction with a large feed of milk. The latter investigator suggested that the mechanism by which this disease is produced may be as follows:

When infective material is present in the abomasum after the ingestion of an abnormally large feed, the casein in the feed renders the acid secretion of the abomasum inert for a period of time sufficient to allow *Cl. welchii* type D to proliferate in the abomasum and to produce a toxin. Progressively increasing numbers of organisms pass out and the chyme becomes progressively more toxic. The toxin is activated in the small intestine and it is possible that there is further proliferation and toxin production as the ingesta traverse the small intestine.

Shaw, Muth and Seghetti[10] were unable

[7]Oxer, D. T.: "Pulpy kidney" or acute infectious enterotoxemia of suckling lambs due to *B. ovitoxicus* (Bennetts). Australian Counc. Sci. & Ind. Res. Pamph. 35 (1932).

[8]Gill, D. A.: Report on pulpy kidney disease of lambs: an acute enterotoxemia of bacterial origin. Vet. J., lxxxix (1933), pp. 399-407.

[9]Roberts, R. S.: Braxy-like diseases of sheep. Vet. Rec., 1 (1938), pp. 591-604.

[10]Shaw, J. N., Muth, O. H., and Seghetti, L.: Pulpy kidney disease in Oregon lambs. Oregon Agr. Exp. Sta. Bul. 367 (1939).

TABLE II—Effect of Filtrates and Toxins on Blood Sugar

Animal	Substance Injected	Dose	First Symptoms	Death	Blood Sugar (Mg. Per Cent)				Urine Sugar
					Pre-Inject.	1st Post-Inject.	2nd Post-Inject.	3rd Post-Inject.	
Rabbit 1	Intestinal filtrate 125	0.4 cc.	20 min.	35 min.	143	268 (autop.)	———	——	Neg.
Rabbit 2	Intestinal filtrate 125	0.15 cc.	None	Lived	154	260 (3½ hrs.)	———	———	———
Rabbit 3	Intestinal filtrate 126	0.1 cc.	1 hr.	Lived	154	154 (1½ hrs.)	———	———	———
Rabbit 4	Intestinal filtrate 127	0.05 cc.	20 min.	3 hrs. 12 min.	121	229 (1 hr. 40 min.)	242 (3 hrs. 10 min.)	———	Trace
Rabbit 5	Toxin + pancreatin	0.2 cc.	20 min.	3 hrs. 42 min.	114	356 (1 hr.)	292 (2½ hrs.)	———	Pos.
Rabbit 6	Dried toxin + pancreatin	24 mg.	13 min.	1 hr. 33 min.	116	347 (1 hr.)	———	———	Pos.
Rabbit 7	Toxin	1.0 cc.	18 min.	43 min.	197	412 (autop.)	———	———	Neg.
Rabbit 8	Toxin	1.0 cc.	13 min.	1 hr. 30 min.	128	330 (1 hr.)	———	———	Neg.
Rabbit 9	Toxin + normal int. filt.	0.5 cc.	———	1 hr. 30 min.	186	382 (autop.)	———	———	———
Rabbit 10	Toxin + normal int. filt.	1.0 cc.	———	6 min.	219	386 (autop.)	———	———	———
Rabbit Control	Normal intestinal filtrate	1.0 cc.	None	Lived	127	152 (30 min.)	145 (2½ hrs.)	———	———
Rabbit Control	Normal intestinal filtrate	1.0 cc.	None	Lived	114	142 (1 hr.)	180 (2½ hrs.)	———	———
Rabbit Control	Sterile broth	1.0 cc.	None	Lived	123	167 (1 hr.)	119 (2½ hrs.)	———	———
Rabbit Control	Sterile broth	1.0 cc.	None	Lived	142	160 (1 hr.)	161 (2½ hrs.)	———	———
Lamb 134	Toxin + normal int. filt.	4.0 cc.	None	Lived	79	86 (30 min.)	93 (3½ hrs.)	———	———
Lamb B	Intestinal filtrate 105	4.0 cc.	3 hrs. 20 min.	5 hrs. 5 min.	107	120 (30 min.)	150 (3½ hrs.)	231 (autop.)	———

to produce pulpy kidney disease with cultures given *per os* following sodium bicarbonate solution or included with a large feed of milk. Neither did they succeed when toxic cultures were introduced directly into the duodenum after laparotomy.

In view of the infrequency at which toxemia and death have been experimentally produced by the enteral route, it is apparent that the processes that allow the toxins to be produced and absorbed in the field cases are not understood. Successful reproduction of so-called enterotoxemias by this route would be of value in proving the etiology of overeating and similar conditions as well as an aid in the study of the processes involved. With this end in view, attempts were made to produce the overeating syndrome with suspected etiologic agents introduced into the alimentary canal.

One lamb (106) which was on a ration containing 0.5 lb. of corn, was given six daily 15-cc. doses of 24-hour cultures of a *Cl. welchii* strain isolated from a lamb. No symptoms developed.

Four laparotomies were performed.* In one, 100 cc. of a toxic intestinal filtrate was injected into the small intestine; in the second, 45 cc. of a 24-hour culture of three strains of *Cl. welchii* of ovine origin were injected into the small intestine; a third received 50 cc. of a toxic intestinal filtrate injected into the colon; the fourth received 50 cc. of an activated toxin injected into the ileum. Symptoms did not develop in any of the lambs and recoveries from the operations were prompt. Blood-sugar determinations were made on the third and fourth lambs. No rise in the level occurred.

Eight yearling lambs have been given cultures of *Cl. welchii* included in large feeds given by stomach tube after the administration of 3 to 5 cc. of 10 per cent copper sulfate to close the esophageal groove. It was hoped to direct the greater part of the feed into the abomasum. Two other lambs were given the same treatment, but without cultures. Death was produced in three of the lambs which received cultures with the feed.

Lamb 45 was given 2 liters of skimmed milk in which was mixed 700 Gm. of corn meal and 225 cc. of a 24-hour broth culture of strain 96 *Cl. welchii.* The mixture was administered at 9:15 a.m. At 7:00 p.m. the lamb refused to eat a little hay that was offered. Death occurred during the night. It was estimated that the lamb lived about 15 hours after the feed was administered. On autopsy the lesions were found to be congestion of the subcutaneous vessels, two irregular subpleural hemorrhages in the dorsal thoracic wall, congestion of the lungs, a few petechial hemorrhages in the thymus, epi- and endocardial hemorrhages and a small hemorrhagic area in the mucosa of the abomasum. Pancreatin was added to filtrates prepared from the contents of the rumen, abomasum and small intestine and was incubated for one hour at 37 C. before rabbit inoculation. The abomasum filtrate in 0.75-cc. dose killed in five hours; the other two filtrates were nontoxic.

Lamb 210 was given 500 Gm. of corn meal in 1,500 cc. of water in midafternoon. The next morning the lamb was scouring. At 9:00 a.m. another feed of the corn meal and water was given and to this was added 200 cc. of a 24-hour broth culture of strain 96 *Cl. welchii.* The diarrhea continued throughout the day, but no other symptoms were observed until 7:15 p.m. At this time the animal was definitely weaker, but still could stand or walk about the pen. At 10:00 p.m. the animal was dead. It was estimated that death occurred one hour previously, or 12 hours after the culture was given. An autopsy was made immediately. Approximately one quart of clear fluid was present in the thoracic cavity; the pericardial sac was distended with partially clotted fluid. Edema of the lungs, numerous endocardial hemorrhages, petechial hemorrhages of the thymus and congestion of the vessels of the serosa of the small intestine and of the mucosa of the ileum were other lesions encountered. Urine obtained from the bladder did not contain sugar qualitatively. Rabbits were

*J. Farquharson and H. W. Johnson assisted in the surgical procedures.

inoculated in 0.5-cc. doses with filtrates prepared from the contents of the rumen, abomasum, small intestine and cecum-colon. The small intestine filtrate killed in five minutes, the cecum-colon filtrate killed overnight, and the others were nontoxic.

Six hundred grams of corn meal in 1,500 cc. of water was given to lamb 178 at 3:30 p. m. A similar feed of corn meal, but containing 200 cc. of a 24-hour broth culture of *Cl. welchii* strain 96, was given on the following morning. Scouring was the only symptom shown throughout the day. On the second morning at 8:30 a third feed of the corn meal was given and to this was added 40 cc. of a 24-hour culture of the same strain of *Cl. welchii*. The diarrhea continued. By 5:00 p. m. the lamb was less active and became steadily weaker in the evening. During the 15 minutes preceding death, the animal struggled. The head was drawn back and there were short periods of forceful movement of the legs. Death occurred at 10:15 p. m., or 13 hours and 45 minutes after the last feed.

The autopsy was made immediately. Lesions were a slight increase in pericardial fluid, numerous small hemorrhages in the mucosa of the abomasum, and congestion in the wall of the small intestine throughout its length. No hemorrhages were observed in the heart, thymus, or serous membranes. Sugar was not found in the urine. Filtrates prepared from the contents of the abomasum, small intestine and cecum were nontoxic for rabbits. The blood-sugar level at the time of the last feed was 70.6 mg. and 74.0 mg. 45 minutes prior to death.

The lambs which did not die were fed and treated in a manner similar to that given the three cases just discussed. Although scouring usually resulted from the feeding of corn meal and water, other symptoms did not develop when cultures were added to the feed.

In the opinion of Roberts,[9] it would be necessary to maintain a state of lowered acidity in the abomasum for a period of time, possibly only a few hours, to provide conditions favorable for the growth of the *Cl. welchii* organisms and the production of their toxin. It is possible that that condition was not obtained in the lambs which did not die. However, from these trials and field observations it seems logical to assume that the mechanism is not entirely explained on the basis of Roberts' theory. When toxic intestinal filtrates or culture toxins were introduced into the intestinal tract of apparently normal lambs in the laparotomy trials, there was no apparent evidence that they were absorbed. In the feedlots it has frequently been noted that scouring precedes deaths from overeating.[11] Many of the lambs which died of overeating, showed an enteritis on autopsy. It is possible that an altered state of the intestinal mucosa is necessary before absorption of the toxin can occur.

SUMMARY

The majority of the filtrates prepared from the intestinal contents of overeating cases were toxic for laboratory animals, confirming previous work.

The toxin produced in culture by *Clostridium welchii* strains isolated from overeating cases was greatly increased in potency by normal intestinal filtrate and by pancreatin. The potency of the activated toxin compared favorably with that of the intestinal filtrates from overeating cases. Like effects were not produced by normal abomasum filtrates or pepsin.

A marked rise in the blood sugar resulted when toxic intestinal filtrates, culture toxin or activated toxin were injected intravenously. Sugar was frequently present in the urine of field cases and in the animals inoculated intravenously.

Death resulted in three out of eight lambs which were given cultures of a strain of *Cl. welchii* isolated from an overeating case, in conjunction with a large feed of grain or milk.

[11]Newsom, I. E., and Thorp, F. Jr.: Lamb diseases in Colorado feedlots. Colorado Exp. Sta. Bul. 448 (1938), p. 27.

The Bacterial Content of Goat Milk*

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THE DAIRY-GOAT industry in Michigan has developed rapidly during the past decade. Goat's milk is used widely for human consumption, in both fluid and dehydrated forms, yet there is very little information available dealing with its bacteriology.

This study was undertaken in coöperation with Michigan dairy-goat owners to determine the incidence of mastitis (infectious and noninfectious) in dairy goats, the effect of udder infection on the quality (chemical and bacteriologic) of the milk secreted, and to determine the physical condition of the udders and the milk produced.

METHOD

Composite and individual half samples were aseptically collected from each lactating goat in four herds and iced until they reached the laboratory, where they were subjected to the various tests. Composite samples were found to yield the true status of each animal. Therefore, only composite samples were collected from each goat in the remaining eleven herds. The results of testing the milk from 380 goats in 15 herds are presented. Included in this study were goats ranging in age from 1 to 11 years, goats which were in all stages of lactation from the first to the tenth lactation, and some that had given milk ranging over a period from 1 to 24 months since the last kidding.

Physical examinations were made of each udder and strip-cut tests were made each time the milk samples were collected for test.

The tests employed to determine the bacteriologic quality of goat's milk were (1) standard plate count, (2) methylene-blue reduction tests and (3) resazurin reduction test. In reading the resazurin test results, any sample pink after one hour of incubation at 37 C. was called class 2 milk; if it was white or completely decolorized at the end of one hour, it was called class 3 milk; and if it remained purple, it was designated as class 1 milk. The chemical and cellular quality was noted by determining the percentage of chloride, the pH and the leucocyte content of the milk. Blood-agar culture and the microscopic test (microscopic examination of properly collected milk samples incubated at 37 C. for 12 to 15 hours) were used to determine the incidence of infectious mastitis among the lactating goats tested.

RESULTS AND DISCUSSION

The total of 380 goats in 15 herds were checked for mastitis. Nine, or 2.3 per cent, of the animals had a streptococcic infection of the udder as determined by the microscopic test and blood-agar culture of the milk. Five others, or 1.3 per cent, had a staphylococcic infection of the udder as determined by isolation of the staphylococci from the blood-agar plate. An additional 2, or 0.5 per cent, were found to have noninfectious mastitis as indicated by physical changes in the character of milk produced and in the udder, with no infectious agent present. The infected goats were not representative of any age groups; some of them were 2 years old and the rest were older. The remaining 364, or 95.9 per cent, of the lactating goats in the herds checked were free of mastitis.

Repeated testing of milk samples from each individual half of the udder and of composite samples of goats in four herds indicated that in so far as the microscopic test, blood-agar culture, bacteria count, methylene-blue and resazurin reduction tests were concerned, it was only necessary to check composite samples from each goat to learn the true status of that animal with respect to the above tests. Individual half samples were necessary to obtain results with any degree of accuracy if the samples were to be used to determine the percentage of chloride, the pH and the leucocyte count of the milk, since dilution with milk from the opposite half might obviate any abnormality in one half of the udder.

The distribution of samples among the various classes of the tests employed (table I) shows close agreement with the results obtained upon testing similar samples of

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milk from cows.[1]

If 0.16 per cent and less chloride in milk is considered as normal, as is customary in cows,[2] we find that 5 per cent of the goats with no udder infection had an abnormal amount of chloride in their milk, while none of those with staphylococcic mastitis yielded milk with a high chloride content, but the milk of 30 per cent of those with streptococcic mastitis had a high chloride content. Five per cent of those with no udder infection were positive to the thybromol test (pH); 40 per cent of those with staphylococcic mastitis and 30 per cent of those with streptococcic mastitis reacted to this test. The normal leucocyte content of milk was taken as 1,000,000 and less per cubic centimeter, similar to that of cow's milk.[3, 4] On this basis 5 per cent of those with no udder infection, 50 per cent of those with staphylococcic mastitis and 50 per cent of those with streptococcic mastitis had a high leucocyte count. These results indicate that the chloride test, the pH and leucocyte count are of very little value in detecting the presence of udder infection, even though they do accurately reveal the percentage of chloride present, the pH and the leucocyte content of the milk.

The physical condition of the udder of each goat checked was determined by careful physical palpation according to the technic of Udall and Johnson.[5] The data are presented in table II. Ninety-two per cent of the goats without mastitis had an udder which was called normal on physical examination, while an additional 4 per cent had atrophied portions of the udder, and 4 per cent more had indurated udders. These physical changes were the result of various noninfectious agents causing injury to the udder. In the case of the goats with staphylococcic infection of the udder, only 20 per cent had normal udders, while only 7 per cent of those with streptococcic mastitis had udders that were normal on physical examination. Although only a few cases of udder infection in goats have been studied, the data presented indicate that the physical condition of the udder usually changes when infection is present, and the majority of those goats with no mastitis (92%) have normal udders.

The strip-cup test or physical appearance of the milk produced bears very little relation to the presence or absence of udder infection, since 0.5 per cent with no infection and a similar percentage of those with staphylococcic mastitis yielded flakes in their milk, while only 5 per cent of those with streptococcic mastitis had flakes.

The bacteriologic quality of the milk produced with udder infection and with no udder infection was determined by the methylene-blue and resazurin reduction tests and the standard plate count (table III). The results in each animal varied slightly from one sampling to the next.

Of the goats with no udder infection, 97.5 per cent gave a milk of class 1 methylene test and the remaining 2.5 per cent produced class 2 milk. The goats with noninfectious mastitis consistently produced class 2 milk during the course of the mastitis, but later their milk was again of class 1 quality. No noninfectious mastitis-affected animal produced milk of class 3 or 4. The staphylococcic infection of the udders definitely reduced the quality of milk produced, since only 56 per cent of the goats thus affected produced class 1 milk; the remainder produced milk of poorer quality as indicated by the results, since 40 per cent produced class 2 and 4 per cent class 3 milk, with none of class 4. Streptococcic infection was even more detrimental to the quality of milk, since only 40 per cent of the goats so infected produced class 1 milk, while an additional 40 per cent yielded class 2 milk and 10 per cent each a milk of classes 3 and 4.

The resazurin test results were similar,

[1]Bryan, C. S.: The relation of streptococcic mastitis to certain phases of milk sanitation. J. Milk Technol., ii (1940), pp. 32-40.

[2]Hucker, G. J.: Mastitis: The incidence and detection of sub-clinical streptococcus mastitis. New York Agr. Exp. Sta. Tech. Bul. 199 (1932).

[3]Bryan, C. S.: The microscopic diagnosis of infectious mastitis. Vet. Med., xxx (1935), pp. 149-156.

[4]Plastridge, W. N., *et al.*: Infectious bovine mastitis. 3. Methods of control. Storrs (Conn.) Agr. Exp. Sta. Bul. 197 (1934).

[5]Udall, D. H., and Johnson, S. J.: The diagnosis and control of mastitis. Cornell Agr. Exp. Sta. Bul. 579 (1933).

TABLE I—Percentage of Milk Samples, from Goats with and Without Udder Infection, Occurring in the Various Classes of Chemical and Cellular Tests Employed

Infection of Udder	Per Cent of Chlorides		pH		Leucocyte Content*			
	0.16 and Less	More Than 0.16	6.4 to 6.7	More Than 6.7	Less Than 250T	250T to 500T	500T to M	More Than M
None	95	5	95	5	45	28	22	5
Staphylococcic	100	0	60	40	10	10	30	50
Streptococcic	70	30	70	30	0	10	40	50

*T = thousand; M = million.

TABLE II—Percentage of Goats with and Without Udder Infection Having Physical Changes in the Udder and Milk Secreted

Infection of Udder	Physical Examination					
	Udder				Milk	
	Indurated	Atrophied	Hypertrophied	Normal	Flakes	Normal
None	4	4	0	92	0.5	99.5
Staphylococcic	28	27	25	20	0.5	99.5
Streptococcic	53	30	10	7	5.0	95

TABLE III—Percentage of Milk Samples, from Goats With and Without Udder Infection, Occurring in the Various Classes of the Quality Tests Employed

Infection of Udder	Meth. Blue				Resazurin			Standard Plate Count				
	1	2	3	4	1	2	3	Less Than 100	100 to 200	200 to 500	500 to 1,000	More Than 1,000
None	97.5	2.5	0	0	94	6	0	44	12	30	10	4
Staphylococcic	56	40	4	0	40	50	10	10	40	25	13	12
Streptococcic	40	40	10	10	37	40	23	10	10	10	20	50

but the data indicate the resazurin test to be more sensitive than the methylene-blue test, since only 94 per cent of the disease-free goats produced class 1 milk as compared with 97.5 per cent in the methylene-blue test, 40 per cent of those with staphylococcic mastitis as compared with 56 per cent class 1 in the methylene test, and 37 per cent of those with streptococcic mastitis produced a resazurin class 1 milk as compared with 40 per cent producing milk of class 1 methylene-blue test.

The bacteria counts obtained were classified according to the number of bacteria present, similar to the manner reported for cows,[1] in order that a comparison might be made. Only 4 per cent of the goats with no mastitis produced milk with a count of more than 1,000, while 12 per cent of those with staphylococcic mastitis and 50 per cent of those with streptococcic mastitis yielded milk with a count of more than 1,000. It is evident that staphylococcic infection of the udder causes an increase in the total number of bacteria present in the milk produced, and that streptococcic infection causes a marked increase of the bacteria count. Forty-four per cent of those with no udder infection had a bacteria count of less than 100. When infection of the udder

was present, only 10 per cent produced milk with a bacteria count of less than 100. The significant point is that almost one half of the goats with no udder infection produced milk with a bacteria count of less than 100, and only a few had a count of over 1,000, while of those with udder infection only 10 per cent produced milk with a count of less than 100. The age, month of lactation, number of previous lactation periods and breed did not influence the quality of the milk produced. The results of examination of milk from goats with no udder infection, but whose udders were indurated as a result of previous injury, did not differ from representative data of noninfected goats whose udders were normal upon physical examination.

SUMMARY

Of the milk samples from 380 goats in 15 herds, 9, or 2.3 per cent, had streptococcic mastitis; 5, or 1.3 per cent, had staphylococcic mastitis and 2, or 0.5 per cent, had noninfectious mastitis. Three hundred and sixty-four of them, or 95.9 per cent, had neither infectious nor noninfectious mastitis. The chloride determination, pH test and leucocyte count of milk can not be used as tests to determine udder infection even if they do accurately determine the chemical and cellular content of the milk.

Ninety-seven and one-half per cent of the noninfected animals gave milk of class 1 methylene blue quality, 94 per cent of class 1 resazurin test quality, and only 4 per cent had a bacteria count of over 1,000. Considering those goats with staphylococcic mastitis, 56 per cent gave a class 1 methylene blue milk, 40 per cent class 1 resazurin milk, and 12 per cent had a bacteria count of more than 1,000. The quality of milk produced by the goats with streptococcic mastitis was greatly reduced, for only 40 per cent gave class 1 methylene-blue milk, 37 per cent class 1 resazurin milk, and 50 per cent yielded milk with a bacteria count of more than 1,000.

Observations on Bacterin Treatment of Salmonella Typhi-Murium Infections in Chinchillas*

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WHAT IS BELIEVED to be the first authentic case of paratyphoid infection (*Salmonella typhi-murium*) recorded in chinchillas (*Chinchilla laniger*) was presented at this laboratory on September 15, 1939.

The infection was present in a herd in which animals are managed for various owners. About 200 animals were being ranched at this particular farm and, from a bystander's point of view, the animals were grouped in pools in an arrangement whereby only a few investors owned any particular animals, but rather had ownership in one or more of these breeding groups. Due to the responsibility vested in the rancher as custodian of such valuable animals, considerable hesitancy was naturally evidenced toward the recommendation for immunization of the herd by bacterin treatment. Since no knowledge of the reaction of these animals to bacterin treatment was available, we also were reluctant to make any broad recommendations. However, following isolation of the causative organism and the making of a diagnosis, recommendations for prophylactic treatment to the herd were given. Results of these attempts at immunization are given in a later paragraph.

ETIOLOGY

The organism consistently isolated from 32 of the 44 animals under study is a short, gram-negative, motile rod producing acid and gas from dextrose, levulose, galactose, arabinose, maltose, mannitol and sorbitol. There is no action on lactose, sucrose, raffinose, salicin or adonitol. The antigenic formula as determined by the late Dr. Fritz Schiff is IV:i:1.2.

This organism is pathogenic for mice, canaries and guinea pigs, as well as for chinchillas.* As stated by Dr. Schiff in his communication, "Cultures with the pure IV antigen, as in this case, ordinarily were obtained from pigeons or from other animals infected by pigeons." The history of the ranch with respect to pigeons showed that none was kept in the vicinity. Of trapped rodents including six common house mice, one wild white-footed mouse and a fox squirrel, four of the domestic species of mouse were found infected. This finding does not necessarily account for the origin of the infection, but it undoubtedly is concerned with its spread.

TYPICAL COURSE OF THE INFECTION

In adult animals the course of this disease was ordinarily acute, with death following within 48 hours of the first noted clinical symptoms. Numerous exceptions to the above course were observed in which a more chronic disease of several days' or weeks' duration was evidenced. Lesions in some of the carcasses examined indicated a chronic infection. In the young animals the peracute form with sudden death was common. While we were not in a position to make such observations or tests, we were informed by the rancher and his attendants that affected animals responded to the treatment and several sick animals made complete recovery. Other than these treated exceptions, it is believed that the disease results in 75 to 80 per cent mortality.

Young animals littered by immunized animals gave evidence of only a passive immunity which was soon lost following weaning.

PATHOLOGY

Adult animals which had shown a chronic

*From the Fish and Wildlife Service, U. S. Department of the Interior.

*Cultures of this organism have been submitted to the American Type Culture Collection at the Georgetown University School of Medicine.

Fig. 1. Colony house on modern chinchilla ranch, showing arrangement of nest boxes and exercise pens for mated pairs and their litters.

infection presented a characteristic picture of paratyphoid infection with enlarged and hemorrhagic Peyers' patches, multiple small white foci in the liver and spleen, and marked icterus of viscera and adipose tissue. Fibrinous peritonitis with adhesions is of common occurrence.

Peracute cases showed less general involvement, with congestion of the intestinal mucosa and inflamed Peyers' patches. In many instances in young animals very few gross lesions were found. Three cases of infection in mature animals showed cutaneous lesions in the form of abscesses. The nature and location of these abscesses, in the pectoral region, belly and within the ear, indicated that wound inoculation was the probable mode of infection in these particular cases. In two of the cases of abscessation, cultures of the Salmonella organism were obtained direct from the abscesses.

IMMUNIZATION

Since chinchillas are polyestrus, a cross section of the herd at any time would show all stages of gestation from the virgin and parturient animal to females at full term. Speculation as to possible injury, from handling and loss due to abortion produced by shock of treatment, proved a constant deterrent to progress on the problem.

A group of open females and old males was selected to serve in a test of the value of autogenous bacterin treatment for this species. Due to their high individual value, we were restricted to the use of only four animals for this test.

Of these four, three were given subcutaneously a phenol-killed suspension containing 200 millions of the Salmonella organism per cubic centimeter. The initial dose used of 0.5 cc. was followed by two 1-cc. doses at five-day intervals. On the seventh day following the final bacterin treatment, each of the four animals was given intraperitoneally 0.1 cc. of a suspension of living organisms with a count approximately that of the bacterin. The control animal was observed to be ill within 24 hours and died within 48 hours. Two of the bacterin-treated animals also died after intervals of seven and eight days, respectively, following inoculation with the live organism. The fourth animal, apparently safely immunized, survived the massive dose after showing only a short period of discomfort and inappetence. Pure cultures of the specific organisms were recov-

ered from the three dead animals. Realizing that the infective dose given these animals in the test was many times that of a probable natural exposure, the rancher was finally emboldened to attempt general bacterin treatment of the herd. For the first group, only males and nonpregnant females were injected. Following successful completion of the series of injections, immunization in early stages of pregnancy was attempted. When unfavorable results were not observed in any of the animals as a result of this treatment, the management decided to immunize all classes of animals including those very close to term. Again only favorable results were obtained and a complete herd immunization was instituted. About six months elapsed between the time of the first tests and the complete herd treatment. During this time numerous normal litters were born and no breaks had occurred in bacterin-protected animals.

Losses following herd immunization were restricted to recently weaned untreated animals.

The questions of the advisability of bacterin treatment of unweaned young, the duration of immunity conferred by the bacterin treatment and the probability of carrier animals in the herd now became of major importance. Answers to these questions were found in the following tests: Young animals from a few days of age onward to maturity to include all animals on the ranch were successfully treated by the use of graduated doses of the bacterin. The remaining original test animal survived a second challenge injection of living organisms six months after the original immunizing treatment. Finally, fresh droppings from ten pairs of animals which had lost their litters due to Salmonella infection were used in the preparation of inoculum for test mice. The mouse test as well as the bacteriologic plate test proved that these animals were not carriers of the paratyphoid infection.

At a later date Mr. Karl Hagen, bacteriologist of the Chinchilla Research Laboratory, working at the ranch, made two complete surveys of all of the animals in the herd and was unable to locate any carriers by bacteriologic methods.

A stained antigen was prepared for use in the conduct of whole blood-agglutination tests and, when used on a small group of animals, showed varying degrees of reaction with a lowering serum titer in animals as elapsed time following immunization increased. From the tests conducted, it was determined that a carefully standardized antigen of this type could be successfully used for the detection of sensitized animals in a herd where bacterin treatment had not been instituted, and that it also could be of service in the detection of animals of low titer in immunized herds, indicating probable susceptibility.

Study of the litter records of females immunized during pregnancy revealed some interesting facts with regard to survival of the young. On the basis of 30 litters produced in eight months following herd immunization, the average litter size showed a decrease of 0.6 per cent when compared with the corresponding period in the healthy herd.

The normal gestation period of the chinchilla is 152 to 154 days.

The earlier in pregnancy that an animal was immunized, the greater was the chance for survival of the young. Records for the period from January 1 to August 31 (immunization of the herd was completed on August 1) showed that of animals immunized within 30 days before parturition, the survival of litters was zero. When dams were immunized 31 to 40 days before term, 33 1/3 per cent of the litters, with 28.5 per cent of the animals, survived.

When the immunization was completed 51 to 60 days before parturition, 80 per cent of the litters lived, with a 50 per cent survival of the young littered.

When immunization was completed any time prior to 100 days before term, survival of litters was 100 per cent with a 92.8 per cent survival of young.

Nineteen young, born to eleven nonimmunized dams at their first littering, died of paratyphoid infection. On second littering following immunization, the same

eleven dams produced 20 young, of which 16 survived. The four young animals which were lost died of causes other than paratyphoid infection.

SUMMARY

What is believed to be the first authentic case of paratyphoid (*Salmonella typhimurium*) infection in chinchillas is reported in a herd of approximately 200 animals. In the study of this infection 44 postmortem examinations, with culture preparations, were made.

From these examinations 32 specimens were diagnosed as paratyphoid, and the remainder as dietary and miscellaneous disorders.

The antigenic formula of the organism isolated from 32 specimens is IV:i:1.2 and represents a strain ordinarily related to pigeons. No relationship between pigeons and the infected animals could be determined in this instance.

The gross pathology of the disease is described, giving characteristic postmortem appearances. The application of a bacterin treatment and the effects on animals well advanced in pregnancy is described.

CONCLUSIONS

Chinchillas (*Chinchilla laniger*) have been found susceptible to *Salmonella typhimurium* and in cases of herd infection show a comparatively high mortality. Chronic, acute and peracute cases will be observed in a herd of mixed ages and sexes.

Chinchillas respond well to autogenous bacterin treatment and no carrier animals were detected by repeated culture tests of fecal material. Wild rodents may be the source and means of spread of the infection in the herd.

Immunization of the dam and sire early in the period of pregnancy greatly enhances the production of living litters and the survival of the young.

ACKNOWLEDGMENTS

We are indebted to the late Dr. Fritz Schiff of the Beth Israel Hospital, New York City, for determination of the antigenic formula and to Mr. Karl Hagen and to other officials of the Chinchilla Research Laboratory for their permission to publish results of their search for host carriers of the infection and for summarization of the data on survival of litters following immunization.

The Effects of Hyperplastic Endometritis on the Blood Cytology of the Dog*

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HYPERPLASTIC ENDOMETRITIS in the bitch is an inflammation of the mucosa of the uterus caused by a uterine hyperplasia upon which a bacterial infection may be superimposed.[1] For purposes of presentation this disease may be classified into two types which are not well defined but which are recognized clinically. In type 1, the cervix becomes entirely or partially closed from edema or fibrosis and there is an accumulation of pus in the uterus. This type of hyperplastic endometritis is commonly called pyometra. In type 2, the cervix remains more or less open and the uterus is not considerably distended. Clinicians sometimes call this condition metritis.

Recently Khuen, Park and Adler[2] and Haigler and Hawkins[3] described certain characteristic deviations from normal in leucocytes in the blood of dogs suffering from metritis or pyometra. These changes have diagnostic value. Similar studies on the blood cytology of dogs suffering from hyperplastic endometritis have been in progress for a number of years at the Raritan Hospital for Animals and changes in the characteristics and percentages of leucocytes have been used successfully to diagnose this disease. Some observations which have been made on the pathology and on the characteristic changes in leucocytes associated with hyperplastic endometritis are presented here to supplement the data now available in the literature.

METHODS

The blood cytology was studied on all dogs received at the hospital in which hyperplastic endometritis was suspected. Diagnosis was confirmed either by hysterectomy or autopsy. The methods described by Morris, Allison and Green[4] were used in the studies on blood cytology.

RESULTS AND DISCUSSION

Large quantities of pus and fluids frequently accumulated in the uterus in type 1 hyperplastic endometritis, with the result that the abdomen was distended in a manner resembling pregnancy or ascites. For example, as much as three liters of fluid was removed from the uterus of a dog weighing 10 kg. The horns of the uteri of the dogs suffering from this pyometra often were found lying plicated into several folds and the fluid in the uterus was usually thick and reddish gray to creamy white, frequently with an offensive odor. The mucosa of the uterus generally was dark red, soft and spongy, with marked pathologic changes. Figure 1 is a photograph of a uterus taken from one of these dogs. Figures 2, 3 and 4 are photomicrographs of sections of this uterus, and the following is a description by Dr. S. A. Goldberg[5] of its pathology:

> The endometrium is markedly thickened. Most of the endometrial cells show cystic dilation and contain polymorphonuclear leucocytes. There are only a few small endometrial glands remaining. The surface epithelium is thrown into folds forming papillary projections into the endometrial cavity. The cells lining the cystic glands and the surface epithelium are columnar cells with pale-staining cytoplasm and with nuclei arranged next to the basement membrane. The myometrium is thickened by hypertrophied muscle fibers. These fibers are in places separated by hyalinized

*From the Raritan Hospital for Animals and the Department of Physiology and Biochemistry, Rutgers University; a contribution of the Bureau of Biological Research, Rutgers University.

[1]De Vita, J.: Hyperplastic endometritis or so-called pyometra of the bitch. J.A.V.M.A., xcv (1939), p. 50.

[2]Khuen, E. C., Park, S. E., and Adler, A. E. Jr.: The hemogram in the differential diagnosis of metritis and pyometra. No. Am. Vet., xxi (1940), p. 666.

[3]Haigler, S. W., and Hawkins, H. P.: Pyometra in dogs. No. Am. Vet., xxi (1940), p. 743.

[4]Morris, M. L., Allison, J. B., and Green, D. F.: Blood cytology of the normal dog. J. Lab. & Clin. Med., xxv (1940), p. 353.

[5]Goldberg, S. A.: Personal communication.

fibrovascular tissues and by areas of plasma cells.

Frequently in type 2 hyperplastic endometritis the vagina and vulva were swollen and a discharge varying in color from dark brown to light yellow was usually present. The uterus was not greatly extended. In advanced and fatal cases the uterus was dark and the mucosa showed necrotic areas. Occasionally the necrosis extended to the muscular and serous lining of the uterine wall. The wall was thickened and edematous and frequently the serous covering was inflamed.

The grams of hemoglobin and the numbers of erythrocytes in the blood of these dogs were usually within the normal range. Similarly, Khuen, Park and Adler[2] found that the erythrocyte count had no diagnostic significance in dogs suffering from pyrometra and metritis. A comparison between numbers and differentiation of leucocytes in normal adult dogs and those suffering from types 1 and 2 hyperplastic endometritis is made in table I. The values for the normal dog were taken from the data of Morris, Allison, and Green.[4] The averages for dogs suffering from type 1 hyperplastic endometritis are based on observations made on twelve dogs from 2½ to 12 years of age. The total leucocyte count in these animals varied from 30,000 to 150,000 per cubic millimeter of blood. The average total white cell and differential counts for six dogs, varying in age from 1 to 4 years, with type 2 hyperplastic endometritis are summarized in the last two columns of table I. The total leucocyte count in these dogs varied from 12,000 to 30,000. The leucocytosis was not as marked or as prevalent in type 2 as in type 1. Khuen, Park and Adler[2] found little leucocytosis in dogs suffering from metritis, a condition probably similar to type 2 hyperplastic endometritis described in this paper. These authors reported an average of 42,500 per cmm. of blood in 16 cases of pyometra, a condition corresponding to the type 1 endometritis. Similarly, Haigler and Hawkins[3] found an average leucocytosis of 50,022 from a study of 24 cases of pyometra in dogs.

Panhysterectomies were performed on five of the twelve dogs suffering from type

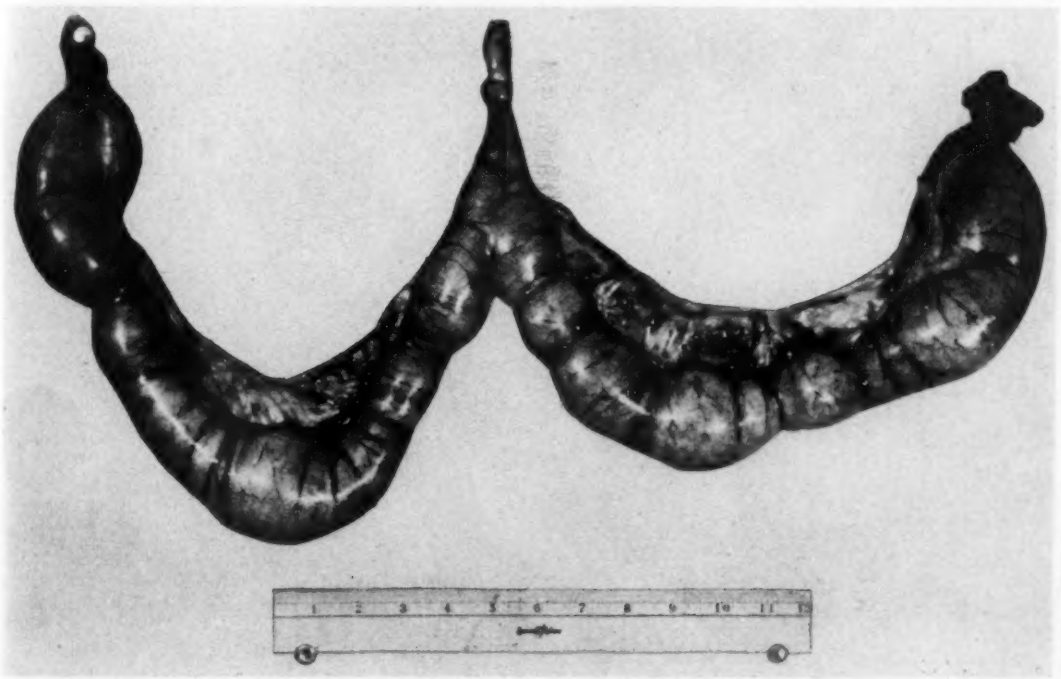

Fig. 1. Uterus taken from a dog suffering from type 1 hyperplastic endometritis.

TABLE I—Comparison Between Numbers and Differentiation of Leucocytes in the Blood of Normal Adult Dogs and of Those Suffering from Hyperplastic Endometritis

	Normal		Hyperplastic Endometritis					
			Type 1		Type 1 Post-Panhysterectomy		Type 2	
	Average Number Per Cmm.	Average Per Cent	Average Number Per Cmm.	Average Per Cent	Average Number Per Cmm.	Average Per Cent	Average Number Per Cmm.	Average Per Cent
Leuc.	11,467		77,010	—	18,950	—	26,125	—
Seg.	7,525	65.27	38,474	49.96	12,555	68.25	13,889	53.17
Lymph.	2,491	21.72	6,430	8.35	2,464	13.0	2,889	11.06
Stab.	753	6.56	26,797	34.80	3,600	19.0	8,509	32.57
Eosin.	623	5.42	117	0.15	190	1.0	262	1.00
Mono.	77	0.67	3,377	4.39	47	0.25	272	1.05
Baso.	—	—	225	0.29	47	0.25	—	—
Juv.	—	—	1,487	1.93	47	0 25	—	—
Myelo.	—	—	103	0.13			301	1 15

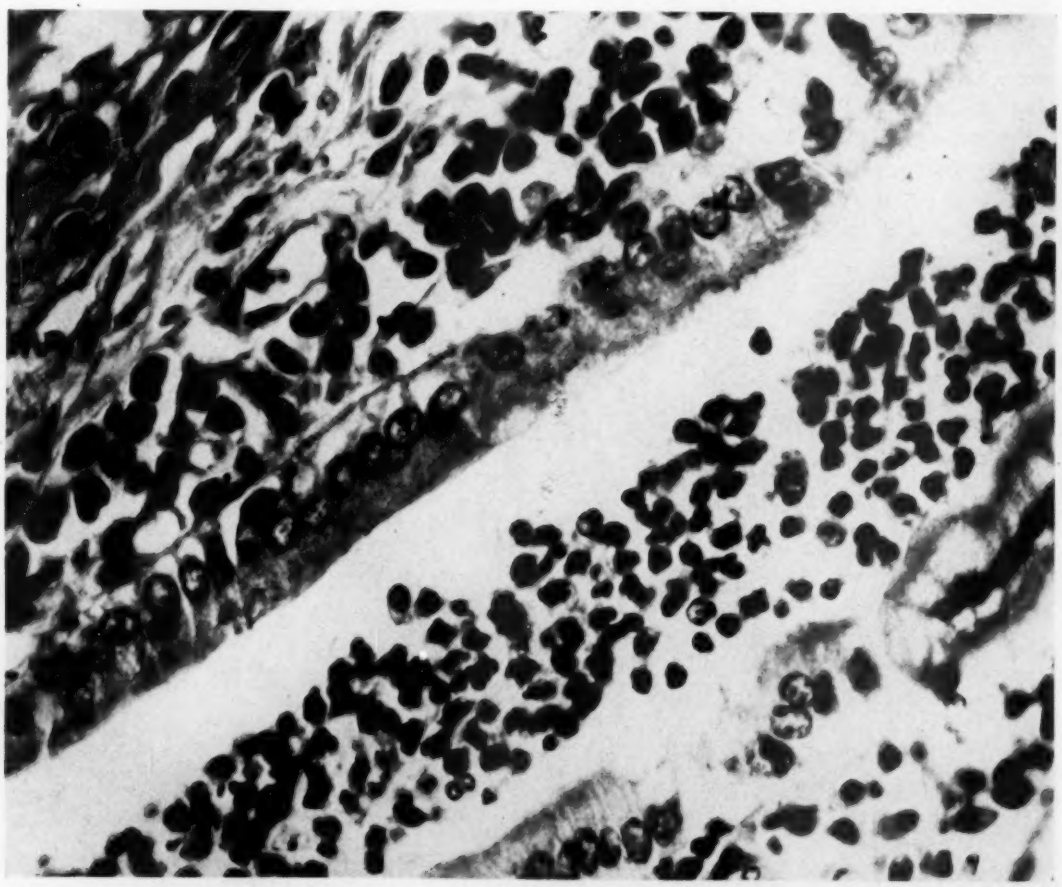

Fig. 2. Section of the uterus shown in figure 1 (x 500). Note tubule lined by columnar cells and filled with polymorphonuclear leucocytes. The interstitial cells are replaced by plasma cells and by hyalinized fibrous tissue.

hyperplastic endometritis. The average counts determined on these dogs approximately six days after the operation are recorded in table I under type 1, post-panhysterectomy. The rapid approach to normal in both the total leucocyte and in the differential count is illustrated by these data.

The leucocytosis found in the data obtained on the dogs suffering from hyperplastic endometritis was due primarily to the increase in polymorphonuclear leucocytes. On the basis of the Schilling classification these data show a "shift to the left," a change which is consistent with those reported for cases of pyometra from other laboratories.[2, 3] The segment/stab ratio for the dogs suffering from this condition was approximately 1.5 to 1 as compared with the normal ratio of 10 to 1.

The most characteristic change observed in the morphology of the leucocytes was the presence of a larger than normal stab cell which stained a light blue with Wright's stain. Figure 5 is a photomicrograph of this stab cell typical of the blood of dogs suffering from hyperplastic endometritis.

The most significant changes, therefore, in the blood cytology of dogs suffering from hyperplastic endometritis were: (1) the marked leucocytosis associated with type 1, (2) the pronounced "shift to the left" in the Schilling differential count with a decrease in the segment/stab ratio and (3) the presence of large, characteristic stab cells associated with both types 1 and 2. During the past ten years blood counts have been made on dogs suffering from various infectious diseases. Never, however, not even in sepsis, has the above blood picture, typical of hyperplastic endometritis, been

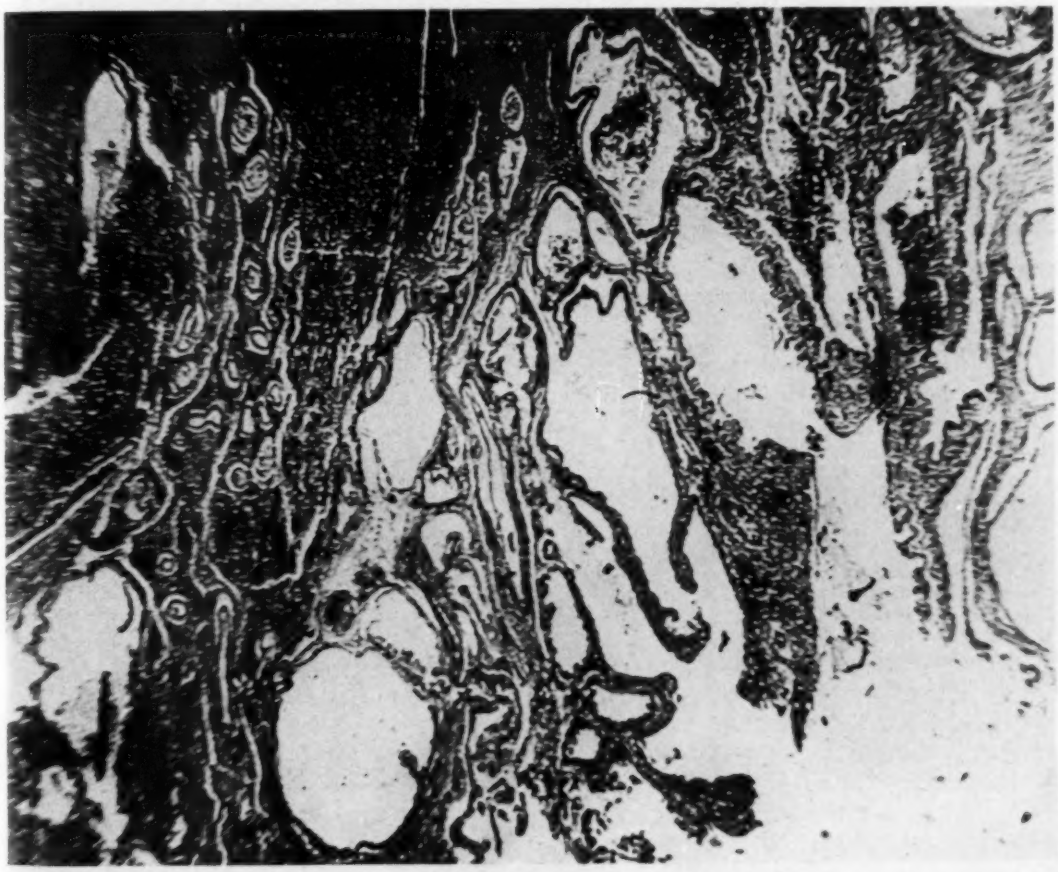

Fig. 3. Section of uterus shown in figure 1 (x 35). Note cystic endometrial tubules and papillary growth at endometrial surface.

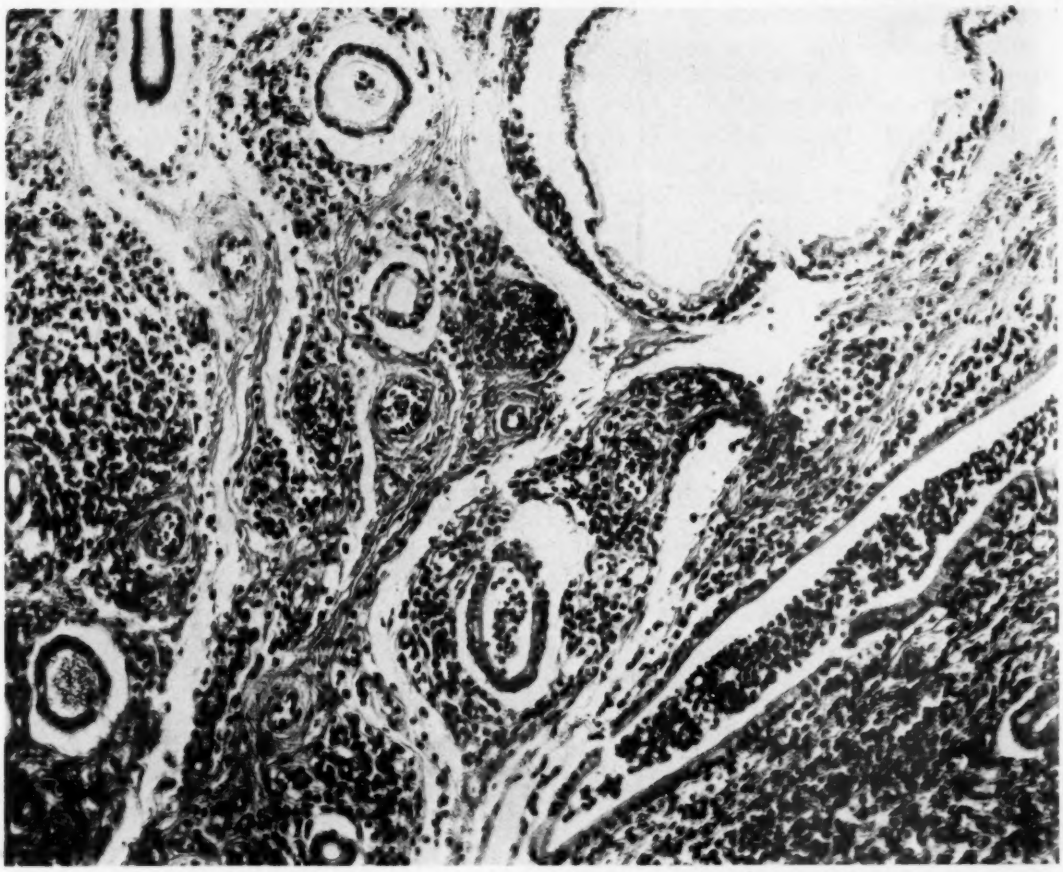

Fig. 4. Section of uterus shown in figure 1 (x 125). Note cystic endometrial tubules filled with polymorphonuclear leucocytes and plasma cells between the tubules.

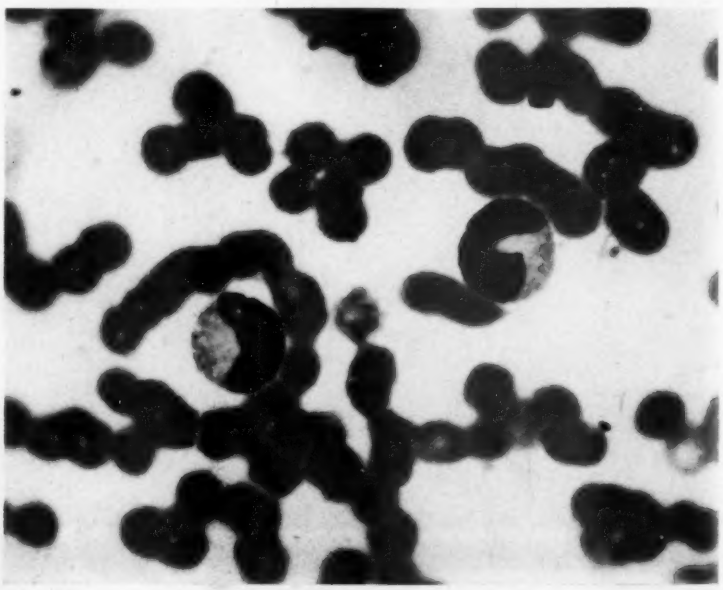

Fig. 5. Photomicrograph of characteristic large stab cell found in the blood of dogs suffering from hyperplastic endometritis.

observed in any other infection. These changes in the Schilling blood count, together with the identification of the characteristic stab cell, have been valuable aids in diagnosing canine endometritis and in differentiating such an infection from ascites, pregnancy or other complicating conditions.

SUMMARY

Some clinical and pathologic observations of hyperplastic endometritis in the dog have been presented. For purposes of presentation the disease was classified into two types: (1) with accumulation of pus in an extended uterus and (2) with little dilatation of the uterus and drainage through an open cervix. A marked leucocytosis accompanied type 1, but less so with type 2. In both types there was a "shift to the left" in the Schilling blood picture with a change from a normal segment/stab ratio of 10 to 1 to approximately 1.5 to 1. This change in the differential count together with the appearance of characteristic large stab cells is valuable in diagnosing this disease in the dog.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. S. A. Goldberg of the Presbyterian Hospital, Newark, N. J., for the photomicrographs of sections of a uterus taken from a dog suffering from type 1 hyperplastic endometritis and for the description of the pathology of the uterus.

Inheritance as a Factor in Poultry-Disease Research*

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THE MODERN CONCEPT of disease recognizes the interdependence of three variable factors: the inherited capacity of the host, the characteristics of the disease-producing agent, and the environment which may materially influence the reaction of the host and the pathogen. In the earliest known writings on disease the idea of contagion recurred from time to time, but only on later rediscovery did it take its proper place in biologic thought. On the other hand, the idea of constitution as a factor in disease was defined clearly in books attributed to Hippocrates and for nearly 2,000 years found repetition in medical literature. Subsequently, appreciation of inherited constitution as a factor in disease phenomena declined and before its revival the concept of a contagious principle in disease causation became dominant largely as a result of Pasteur's brilliant work. A paper by Mendel published in 1865 pointed out that certain characteristics of the garden pea are inherited on a unit basis. This paper was the foundation for the science of genetics and our knowledge of the physical basis of inheritance. However, it is only within the present century that the genetics of the host has found proper standing as a factor in disease investigation. As far as heredity of pathogenic organisms is concerned, practically nothing is known.

To environment, the third general variable in disease, the ancients ascribed both direct and indirect rôles. Consideration of the influence of climate, season, food and other environmental factors upon both the host and the parasitic or infectious agent has, however, persisted with fewer adversities than beset the principles of host and contagious agencies in disease causation.

Biffen's work (1905) on resistance to mycotic stem rust in wheat demonstrated the importance of heredity in relation to an infectious disease in higher plants and laid the foundation for important advances

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in the knowledge of heredity in disease. Although work with plants developed rapidly, study and progress in the same direction with animals were restricted largely because of practical and intrinsic difficulties. The advantages offered by plants, such as self-fertilization, short generation time and the availability of large numbers with limited facilities, are not at the disposal of workers in the animal field.

The evidence acquired in plant genetics suggested that the inherited constitution of the animal should be recognized as an important host variable in various infectious diseases as well as in disturbances associated with obvious structural and functional anomalies. Significant examples of the effect of inheritance of noninfectious pathologic conditions in the animal kingdom are represented by the yellow mouse lethal (Cuenot, 1908), lethal anemia of mice associated with white spotting (Little, 1915), silk worm lethals (Nishikawa, 1931), achondroplasia in cattle (Crew, 1923; Mohr, 1929), cryptorchidism in goats (Lush *et al.*, 1930) and cryptorchidism in swine (McPhee and Buckley, 1934). In poultry heritable deviations from normal or desirable structure and function are seen in the lethal linked with recessive white (Dunn, 1923), creeper fowl (Landauer and Dunn, 1930), congenital loco (Knowlton, 1929), dwarfism (Mayhew and Upp, 1932), stickiness in fowl embryos (Byerly and Jull, 1932), crest in ducks (Rüst, 1932), congenital tremor in chicks (Hutt and Child, 1934), pendulous crop in turkeys (Asmundson and Hinshaw, 1936) and lethal upper mandible mutation (Asmundson, 1936).

Until quite recently evidence of heritable differences in susceptibility to disease among animals was confined to demonstrations of species and racial or varietal differences. Definite evidence of genetic differences in susceptibility among families within species or varieties was, however, provided by Hagedoorn-Labrand and Hagedoorn (1920), Frateur (1924), Wright and Lewis (1921), Roberts and Card (1926), Lambert and Knox (1928), Manresa (1932), Gowen and Schott (1932, 1933), Webster (1923, 1937) and Asmundson and Biely (1932). In the experimental work with various neoplasms and with leucemia in laboratory animals the rôle of hereditary resistance and susceptibility has been repeatedly shown (Tyzzer, 1907; Haaland, 1907; Cuenot and Mercier, 1908, 1910; Murray, 1911; Slye, 1913, 1931; Macdowell and Richter, 1930, and others).

The possibility that a pathogenic organism or virus may change or mutate is fraught with significance. Gowen and Price (1936) suggested that certain viruses have gene-like attributes which are inactivated by both ultraviolet and x-rays. The work of Arkwright (1920) and of many others has demonstrated the importance of bacterial variation or dissociation as represented by smooth to rough alterations.

It becomes obvious that aside from environmental considerations the research worker in animal diseases is faced with the necessity of appraising properly the factors of constitution of the host and the disease-inducing agent and, therefore, should strive for experimental material with a known genetic background.

The necessity of working with host material of known resistance or susceptibility was demonstrated by Webster and Hodes (1939). Highly inbred lines of mice, relatively resistant or susceptible to a standard test dose of *B. enteritidis*, were used as test material. With such inbred mice these workers were successful in obtaining standardized reaction in transmission experiments. Webster (1939) aptly stated: "Studies now under way indicate that the level of resistance which is inherited can be altered by many environmental factors, entirely aside from specific vaccines or sera. Not the least of these factors, for example, is diet." This environmental influence has not been overlooked in numerous other investigations.

GENETIC PRINCIPLES AND IMPLICATIONS

The science of genetics has demonstrated beyond doubt that the fundamental aspects of heredity are relatively simple. It is safe to say, however, that the mechanisms of inheritance still would be a mystery were it not for the existence, in both the plant

and animal kingdom, of easily differentiated allelic pairs of genes. The first aim in the preparation of material for a genetic study is that the genes for the character or trait studied should be present in a homozygous condition. If the characters studied differ only in one or two pairs of genes, which are easily differentiated, it is relatively easy to obtain homozygous material with which to work. Unfortunately, the majority of characters of great economic importance in both plants and animals are dependent on the interaction of many pairs of genes. The genetics of resistance and susceptibility to disease seem to fall into the same category.

Geneticists have recognized the usefulness of inbreeding for establishing uniform families or strains in plants or animals. Such a system of mating automatically brings about a general increase in the genetic purity or homozygosity of the inbred population. The genetic likeness is increased within each inbred family, but at the same time the diversity from family to family is increased, even though these families start from common foundation stock. Inbreeding increases homozygosity for bad as well as good factors and may fix undesirable combinations of genes, with the result that some deterioration usually occurs in those qualities generally found in a good, vigorous stock. The inbreeding does not create the defective traits that appear, but merely uncovers them by bringing to a homozygous condition undesirable recessive genes that were not visible in the parents. Little systematic inbreeding experimentation has been done in large domestic animals. In the small laboratory mammals highly inbred strains are available in mice, rats and guinea pigs. These small inbred laboratory mammals have proved of great value in medical research where comparable material was essential. The use of blind breeding practices in animals only tends to confuse an already complicated situation.

There is enough information available to show that innate resistance or susceptibility to disease exists in breeds and varieties of animals. The problem is to breed these animals in such a way as to establish strains or families whose progeny will react to specific tests with a high degree of prediction. Meanwhile the problem of maintaining viable lines or strains resistant to the more prevalent diseases remains largely unexplored.

MECHANISMS OF NATURAL RESISTANCE

The commonly considered natural barriers against infection with which each functionally and structurally normal individual is endowed include the integument, the secretions and body fluids and the cells comprising the tissues. Obviously these defenses may be subject to intraspecies and individual variations. Webster (1939) pointed out that in certain diseases the inherent capacity for resistance to infection is not as manifest at the portal of entry or in the blood stream as in the specific tissues for which the infection has a predilection, *e.g.*, St. Louis encephalitis in mice. Among species, insusceptibility to some diseases has been variously ascribed to natural antibodies, to lack of suitable nutritive factors or to unfavorable temperature environment.

Gross characters which distinguish one species from another were early assumed to be associated with natural resistance among species. Family and individual susceptibility to tuberculosis have been explained on the basis of inherited subnormal chest development and capacity. Furthermore, later findings justify holding various cellular and physiologic factors accountable for natural immunity among some individuals and lines or families. Rich in 1923 found that in guinea pigs of certain families low and high complement titre bore a distinct relationship to resistance to *Pasteurella suiseptica* as well as *Salmonella choleraesuis*. Casey and Pearce (1931) observed that the pre-inoculation blood cell picture was a highly accurate index of the individual resistance or susceptibility of rabbits to transmissible malignant tumors. Strong and Francis (1940) demonstrated a continuous and precipitous fall in the hemoglobin level of a cancer-susceptible strain of mice, whereas no such decrease occurred in a relatively cancer-resistant strain of

mice. Irwin and Bell (1935) studied natural resistance of rabbits to *Brucella suis* and observed that the low and high bactericidal action of the citrated whole blood of rabbits was closely correlated with susceptibility and resistance to *Br. suis*. The specificity of the natural bactericidins was demonstrated in tests with *Br. abortus* and *Salmonella enteritidis*.

In a study of the blood of pullorum resistant and susceptible chicks, Roberts, Severens and Card (1939) found that the lymphocytes are present in greater number and percentages in the resistant chicks from the eighteenth day of incubation through the third day after hatching. This observation is particularly significant because of the fact that resistance increases rapidly during the first few days after hatching, a process associated with a normal increase in lymphocytes. Ackert and coworkers (1938, 1939) were able to demonstrate that age resistance of the chicken to the fowl nematode, *Ascaridia lineata*, developed concomitantly with an increase of goblet cells in the duodenal mucosa. It also was shown that mucus secreted by the goblet cells contained a factor which inhibited development of the parasite.

Aside from such limited information, the mechanism of inherited immunity in many diseases of animals remains largely unsolved. The situation is even less satisfactory with regard to a knowledge of factors of inherited constitution of pathogenic organisms. However, the development of improved methods of stabilization of bacteria, viruses and other infectious agents, *e.g.*, by low-temperature freezing and drying *in vacuo*, should tend to clarify the influence of factitious environment and of inheritance upon such important characters as invasiveness and virulence. Obviously, the genetic variability can not be measured until the extrinsic variability is known.

DISCUSSION

The results and implications of the work on inheritance in relation to disease in both the plant and animal kingdom can not be lost sight of in the scientific approach to the problems of poultry diseases. Knowledge which has been acquired from studies of inherent constitution in certain noninfectious, infectious and parasitic diseases of poultry serves to emphasize the need for careful appraisal and consideration of constitutional as well as environmental factors. Certainly, the use of experimental material in which variability in hereditary pattern may be limited or fixed within the narrowest possible range will make for more rapid progress.

Chickens and other species of poultry have the advantage of being relatively economical to keep and breed in numbers sufficiently large for the study of genetics in relation to disease. By exposure of unselected stock to a given infection a classification into resistant and susceptible categories is sometimes possible even in a single generation of birds (Lambert and Knox, 1932; Roberts and Card, 1935; etc.). Such natural resistance or susceptibility has been found highly specific; seldom is it less so than immunity acquired through experience with the etiologic agent. An excellent example of such specificity is seen in the work of Gowen and Schott. (See Gowen, 1935). Comparisons of the reaction of genetically different lines of mice to (a) mouse typhoid, (b) pseudorabies and (c) ricin indicated that genetic constitution for resistance is evidently specific for the strain of animal.

Of major importance is the possibility that genetic research will lead to identification of important environmental or extrinsic factors in disease causation. Demonstration of the "milk factor influence" on breast-tumor production in mice was made possible by utilization of pure lines of mice (Bittner, 1936-1939). By reciprocal foster nursing of the offspring of mammary cancer-resistant and mammary cancer-susceptible mice it was shown that the source of milk was more important than the genetic constitution of the individual in the etiology of the breast cancer.

There is also the chance that the specificity of inherent factors of the host may be utilized in the differentiation of somewhat similar diseases, with pathogenic potencies limited to a single species. The

importance of the hereditary factor in disease is stressed in the findings of Webster and Clow (1936). Following the intranasal instillation of St. Louis encephalitis virus into mice, lesions appeared in the brains of susceptible mice as early as three days after exposure, whereas lesions did not appear before the eighth day in resistant stock mice. The titer of the virus drops when recovered from resistant mice and becomes noninfective by the nasal route. Passage in susceptible mice promptly restores its full titer.

In using the terms resistance or immunity, and susceptibility, care in definition is imperative. In so far as the literature will permit interpretation, these terms are relative and do not imply complete immunity or susceptibility. All of the individuals comprising so-called resistant strains of plants or animals are not absolutely immune to specific diseases. Neither will all members of so-called susceptible strains succumb to "standardized" doses of a specific pathogen. The reaction of either immune or susceptible hosts to a specific pathogen depends to a large extent on not one but many environmental variables. No experiment of this type can be completely analyzed unless it lends itself to statistical treatment.

Frequent testing of host material is desirable or even imperative if the degree of immunity is to be maintained or enhanced. A breeding program necessitates continuous careful selection even after a high degree of resistance or susceptibility exists in the stock. It is entirely possible that over a period of time unexplainable innate changes in either the host or pathogen may occur which will lead to contradictory results. This can be minimized in part by following selective breeding procedures with the host together with periodic tests and a stabilizing environment for the pathogen.

The organization of a study for appraisal of inborn resistance to a specific disease should include a program which permits breeding only from unexposed sibs of tested stock and not from the inoculated or otherwise exposed survivors. Webster (1937) stressed the hazards which may result from the practice of breeding from inoculated survivors. It may be difficult to demonstrate that inoculated survivors are free of infection. Adequately unexposed controls must be maintained as a check on all inoculated material. This will be facilitated by a distribution of sibs from the same dam to both control and inoculated groups.

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[A series of reviews by Morgan (1922), Crew (1926), Kozelka (1929), Gowen (1935, 1937), Parker (1937) and Babcock (1939) present excellent discussions and bibliographies on genetics as related to disease in plants and animals.]

A Study of Neoplastic Disease in a Flock of Chickens*

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A SERIES OF experiments with a small poultry flock was begun in our laboratory in the fall of 1937, for a twofold purpose. One object was to make a survey of all cases of neoplasia that developed in a small poultry flock; the other was to attempt experimental transmission of those cases of fowl leucosis and lymphocytoma from which suitable material could be obtained. This article describes the tumors that were found in the flock and the transmission experiments, with one exception. One experiment led to the development of a transmissible lymphoid neoplasm of the chicken, and it is described in a separate report.[1]

Surveys of the incidence of neoplastic disease in poultry flocks have been reported by various investigators. The work of Goss[2] is one of the most comprehensive reports of this type. Numerous reports on the transmissibility of various neoplasms of the chicken have been made by many investigators. However, the available literature contains no account of attempts to transmit the different cases of neoplasia arising spontaneously in the same poultry flock, as was done in this work.

History of the Flock

A flock of 48 chickens was being raised to supply eggs and meat for a small family. The birds were purchased when 1 day old and were raised in a yard where there had been no poultry for four years. The chicks did not have direct contact with other birds, although a few older chickens were being kept on the same premises. Disease had not occurred among the older adult birds. The birds of the flock were crossbred (the result of mating Rhode Island Red females to Barred Plymouth Rock males) and were obtained from a commercial hatchery. All appeared to grow and develop normally. At the end of 110 days (August 25, 1937), however, one chicken was found dead. This bird was brought to the laboratory and was found to be affected with fowl leucosis (case 413). Experimental chickens were inoculated with its blood, with results described in a later paragraph dealing with this case.

The flock was examined on several occasions after the death of this bird and an agreement was reached with the owner to submit to the laboratory for examination any sick birds or birds found diseased at the time they were killed. This program was carried out. Blood smears were made from all the birds of the flock at four intervals during the seven weeks after the first loss from leucosis. One other case of leucosis (case 414) was found and several of the birds developed neoplastic disease (table I). Originally there were 27 males and 21 females in the flock. When the birds were about 5 months old, the first of the cockerels was killed for food for the family. The last male was killed at an age of about 8 months. The last case of neoplastic disease occurred in a hen 2 years and 4 months old. At this time, five hens were killed for meat purposes and found to be apparently normal. The remaining three hens were slaughtered at about 3 years of age. One hen died with a nonneoplastic disease (salpingitis). Most of the losses from tumors occurred before the birds were 10 months old (eight cases).

Description of Cases of Neoplastic Diseases

Case 413. — Erythroblastic Leucosis: The owner reported that he had noticed the bird to be pale and weak the day before its death. A blood smear made at the time of necropsy revealed the presence of slightly immature erythrocytes and many lymphoid cells. Relatively

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[1]Olson, C.: A transmissible lymphoid tumor of the chicken. Cancer Res., i (1941), pp. 384-392.

[2]Goss, L. J.: The incidence and classification of avian tumors. Cornell Vet., xxx (1940), pp. 75-88.

few intermediate cell forms were present. The gross and microscopic changes in the liver, spleen and bone marrow were those of erythroblastic leucosis. An emulsion of bone marrow was prepared and inoculated intravenously into eight cross-bred chickens 29 days old. They were held under observation for 60 days, during which time repeated blood examinations were made. All but one remained negative for leucosis. The exception began to show leucotic changes in the blood 23 days after inoculation and died of erythroblastic leucosis after an illness of 8 days. One chicken developed symptoms of fowl paralysis 42 days after inoculation, and when killed ten days later showed well-developed gross and microscopic lesions of fowl paralysis.

The group of eight chickens inoculated with bone-marrow emulsion were of a different stock from that generally used for experimental purposes and exact data on a comparable control group are not available. Although the development of a single case of leucosis in the group is meager evidence of transmission of the disease, two facts tend to support this conclusion. Leucosis is rare as a spontaneous disease in birds of the age used, and the incubation period and course of the case observed are in accordance with what has been noted by other investigators.[3] Considerable evidence indicates that the agent of fowl leucosis does not produce fowl paralysis,[3] and as fowl paralysis may occur as a spontaneous disease in chickens 71 days of age, the single case found is regarded as unrelated to the inoculation.

The remaining six chickens (M31, M34, M35, M36, M37 and M38) were subsequently inoculated with material from another bird of the flock (case 452). Leucotic bone-marrow inoculum prepared from case 413 was mixed with an equal quantity of glycerin and held in the ice box for eight days. This glycerinated marrow preparation was then inoculated intraperitoneally into four cross-bred chicks 5 to 14 days old and four chickens 55 to 62 days old. All remained negative for leucosis and 50 days later some (M143, M144, M145 and M146) were used in transmission experiments with the transmissible lymphoid tumor developed from case 452.

Case 414.—Erythroblastic Leucosis: At the time of the first blood examination, made August 26, this bird was found to have changes in the blood indicative of fowl leucosis. The bird died three days later and the necropsy and histologic findings confirmed the diagnosis of typical erythroblastic leucosis. On the day preceding death the erythrocyte count was 1.57 million per cubic millimeter and the hemoglobin value 6.7 Gm. per 100 cc. of blood. At this time blood was obtained in citrate solution for intravenous inoculation into experimental cross-bred chickens, one 46 days old and two 12 days old (M39, M41 and M42, respectively). Changes in the blood of M41 were noted six days after inoculation and the picture of the blood 13 days after inoculation was characteristic of erythroblastic leucosis. Blood from this bird (M41) was used as inoculum for other experimental chickens. Only one of the other birds in the first experimental passage developed erythroblastic leucosis. This strain of leucotic agent was carried in serial passage through three series of experimental chickens, involving the use of 14 birds, and in 6 of these erythroblastic leucosis occurred.

The first changes in the blood of the six leucotic chickens were noted at 27 days, and death occurred at 55 days after inoculation (average figures). In five of the cases the disease was identical with that in the original donor chicken. The sixth case was complicated by the presence of a fibrosarcoma in the musculature of the left breast and on the serous surface of the viscera, in addition to the lesions of erythroblastic leucosis. The fibrosarcoma was of variable composition. The tumor contained many relatively large blood spaces, in some of which the blood was coagulated and partially organized. There was considerable variation of the neoplastic fibroblasts, most of which tended to assume a spindle-shaped form with small nuclei, while others possessed large vesicular, anaplastic nuclei. Many multinucleated giant cells were scattered throughout the tumor. In some areas there was considerable clear, intercellular material. The chicken had received its inoculum of leucotic blood in the intraperitoneal cavity. The connection between the fibrosarcoma and leucosis in this instance is somewhat difficult to understand, although one must bear in mind the potentiality of certain strains of the agent of leucosis to produce fibrosarcoma after intermuscular inoculation.[3] This particular strain of leucotic agent was lost in spite of attempts at further passage with two samples of leucotic blood, previously infective, that had been mixed with glycerin and held for 102 and 153 days, respectively.

Case 444.—Hemangioma: The chicken was found dead on October 11, 1937. An examination of the blood made 46 days previously showed no significant changes. A slight relative eosinophilia was noted 39 days prior to death, and 25 days preceding death a moderate, relative heterophil leucocytosis was observed. A swelling of the left foot pad was noted about a week before the bird died, and on the day before death it was weak, dull and listless. The carcass was emaciated. The swelling of the left foot pad was soft and fluctuating and on section was found to be com-

[3]Olson, C.: Transmissible fowl leukosis. A review of the literature. Massachusetts Agr. Exp. Sta. Bul. 370 (1940).

posed of clotted blood and what appeared to be granulation tissue.

The liver was slightly swollen, reddish brown and contained numerous foci of clotted blood under the capsule and deep into the substance of the organ. Similar foci of accumulated blood were present in the kidneys, lungs, heart and spleen. The spleen was enlarged (2 cm. in diameter) and consisted principally of an ancient blood clot. Splenic tissue was confined to a narrow crescentic border along one edge of the mass representing the spleen. The foci of blood in the heart muscle and in the kidney seemed to have a thin though distinct surrounding membrane. The blood in the heart chambers was not clotted.

Pathologic changes could not be noted in a smear prepared from the heart blood. The histology of the tumor was portrayed best in the foci of the heart and lung. The type cell was the angioblast. In some areas these were compact and in others they tended to form small spaces which were filled with blood. The lining of the larger blood spaces was stretched thin. In many of the larger blood spaces the blood was coagulated, and varying degrees of organization were apparent.

Case 447.—Neurogenic Sarcoma: The first symptoms of illness were observed when the chicken was 131 days old and these were indicative of fowl paralysis. The bird was unable to stand, although it was able to move the wings and legs. It remained paralyzed and slowly lost flesh. It was killed 34 days after it became ill. No abnormalities had been observed in repeated blood-smear examinations, except in one instance. Nineteen days preceding death a moderate, relative, heterophilic leucocytosis was noted.

At necropsy a tumor was found near the base of the heart and immediately dorsal and medial to the right thyroid gland. The mass was well encapsulated, very firm and white. It was found to be attached to elements of the right brachial plexus. Some of the tumor, growing in the vertebral canal, caused marked compression of the spinal cord. The main portion of the mass was free of the cervical muscles and connected by a narrow stalk with the part in the vertebral canal. The main tumor mass measured 5x4x2.5 cm. The surface of the tumor was somewhat irregular as there was a tendency towards lobulation of the mass (fig. 1).

Histologically, the tumor was composed of spindle-shaped fibroblastic-like cells with varying amounts of intercellular material at different parts (fig. 2). The visceral organs and other parts of the nervous system were normal.

Case T 60.—Fibrosarcoma: The chicken was 402 days old at the time it was killed for food. For the previous two months it was lame and was becoming emaciated. The musculature of the left leg was markedly swollen and was composed of firm, white, fibrous tissue. The shaft of the femur was roughened and appeared to be infiltrated by the tumor. There was a recent fracture at the distal end of the femur. The tumor consisted of young fibroblastic cells with large vesicular nuclei having one or two nucleoli. The intercellular substance was variable in amounts, and one area stained with a distinct blue tint suggestive of chondromucin. Metastatic tumor foci were found in the musculature of the gizzard and in the wall of the duodenum.

Case 616.—Fibrosarcoma: At the age of 480 days the chicken was found down and unable to stand. It was able to move its feet and struggled violently when restrained for examination. The bird made no sounds, apparently a condition of aphasia, which suggested the existence of a brain lesion. A small, firm mass was palpable in the musculature of the right breast. The chicken was emaciated and did not eat. It was killed on the following day.

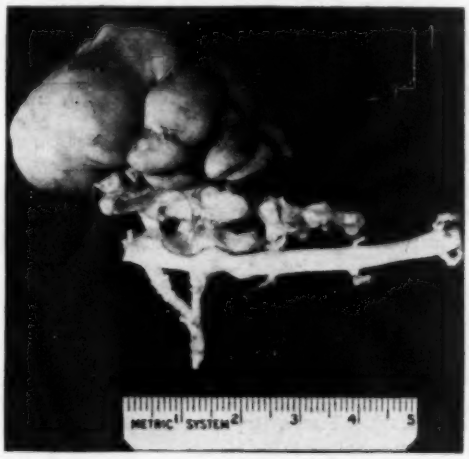

Fig. 1. Ventral view of neurogenic sarcoma (case 447). Note the large tumor arising from the nerve roots of the left brachial plexus. The tumor compressed the spinal cord, extended through the intervertebral foramen and attained its greatest growth at a site removed from the vertebral column.

A small, firm, spindle-shaped tumor was found in the right breast muscle. The only other lesion found was a small, discrete gray area on the ventral aspect of the brain stem adjacent to the optic lobe. On section this proved to be an area of gliosis in which were elements representing metastatic foci of the fibrosarcoma.

Case T60.—Fibrosarcoma: The chicken was denly at an age of 559 days. At necropsy tumor tissue was found scattered on the serous surfaces of the viscera. The ovary was affected, but it was not greatly increased in size. Upon histologic examination the tumor was found to be a carcinoma, probably originating in the ovary.

TABLE I—Neoplastic Disease in Flock of 48 Chickens

CASE*	DIAGNOSIS	AGE IN DAYS	REMARKS
413	Fowl leucosis	110	Transmissible
414	Fowl leucosis	115	Transmissible
444	Hemangioma	156	
419	Lymphocytoma	161	Not transmissible
447	Neurogenic sarcoma	164	
448	Lymphocytoma and embryonal nephroma	167	
452	Lymphoid tumor	205	Transmissible
480	Lymphocytoma	278	
T60	Fibrosarcoma	402	
T61	Lymphocytoma	406	
616	Fibrosarcoma, gliosis	481	
644	Carcinoma	559	
922	Carcinoma	859	

*All females except case 448.

Case 922.—Carcinoma (Carsinosarcoma): The chicken was killed for food, but was found to be diseased and was brought to the laboratory for examination. Before the bird was killed, it was observed that the abdomen was distended. One large and one small mass consisting of multilocular cysts filled with yellow and yellow-brown, semisolid fluid were found in the visceral cavity. The attachment of these masses could not be readily established, though it appeared that they derived their vascular supply from the mesenteric vessels. Smaller implants were scattered over the serous surface of the abdomen. The adrenals were infiltrated with gray tissue. The ovary, spleen, liver and kidneys were essentially normal. The histologic picture of the tumor displayed a mixture of adenocarcinoma and spindle-celled sarcoma tissue. The relative proportion of each type and its character varied in different locations of the tumor tissue (figs. 3 and 4). Jackson[1] has described and adequately discussed a type of mixed tumor composed of epithelial and smooth-muscle elements (carcinoma lieomyomatosum). Careful study was required to differentiate the fibroblasts from smooth-muscle cells. The origin of the tumor might have been the mesoblastic elements of the adrenal. The character of the tumor was quite similar to that observed in some cases of embryonal nephroma and the possibility of origin from misplaced mesonephric tissue exists in this case.

Case 447.—Lymphocytoma and Embryonal Nephroma: This, the only male chicken found to be affected with neoplastic disease, died at the age of 167 days. The bird had been noted to be dull and depressed for a few days before its death. No abnormality was noted in the last blood examination made 36 days previous to death. At necropsy the carcass was emaciated. The liver, spleen and bursa of Fabricius were markedly enlarged and infiltrated with lymphoid cells. Masses of lymphoid cells were located on the peritoneum of the sub-lumbar fossa under the kidneys and adjacent to the keel bone. Upon microscopic examination an area of embryonal tissue was found in the kidney, in which there were imperfectly formed tubules. Material suitable for animal inoculation was not obtained from the case.

Case 480.—Lymphocytoma: The chicken died at the age of 278 days. Examinations of the blood showed no abnormalities (last examination 22 weeks previous to death). Diffusely infiltrating neoplastic lymphoid tissue was found in the liver, spleen, ovary, kidneys and bone marrow. Experimental transmission was not attempted.

Case T61.—Lymphocytoma: Death occurred at the age of 406 days. Neoplastic lymphoid tumor tissue was found involving the serous surfaces of the visceral cavity, the ovary, kidneys and spleen. No experimental chickens were inoculated with the tumor.

Case 419.—Lymphocytoma: At the age of 116 days examination of the blood revealed a state of anemia in which there were many moderately immature erythrocytes, but no very young cells as in leucosis. The condition of anemia disappeared in the course of the next week. However, while it was still present, blood was obtained for intravenous inoculation into four experimental chickens aged 17 days. Each received approximately 0.4 cc. of whole blood. They remained negative for eight weeks, at the end of which this experiment was terminated. Repeated examinations of the blood of the chicken were made and a state of anemia was again manifest eight days preceding death. At the age of 161 days the bird was weak and it was killed. The carcass was emaciated (weight 1,390 Gm.). The liver was enlarged (weight 170 Gm.) and diffusely infiltrated with mottled, grayish tissue which in some areas was coalesced into solid masses up to 1.5 cm. in diameter. The spleen was normal in size (weight 4.75 Gm.) and in color. The other visceral organs (kidneys, ovary, lungs, bursa of Fabricius and intestinal tract) were negative for gross pathology. Histologically the hepatic parenchyma was largely replaced by an extravascular growth of lymphoid cells which were actively multiplying. Very small localized foci of similar neoplastic lymphoid cells were found in the ovary, speen and bursa of Fabricius.

One of the larger tumor masses of the liver was used for the inoculum in an attempt to transmit the tumor to experimental chickens.

[1]Jackson, C.: Incidence and pathology of tumors of domesticated animals in South Africa. Onderstepoört J. Vet. Sci. & Anim. Ind., vi (1936), pp. 1-460.

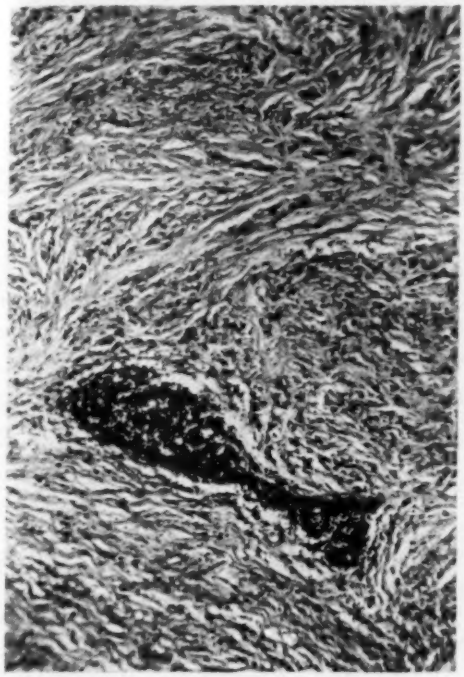

Fig. 2. Neurogenic sarcoma (case 447). The tumor is composed chiefly of irregularly arranged fibroblasts. A focal accumulation of lymphocytes is present in the field (x 100).

The tumor tissue was removed with sterile instruments and placed in a Petri dish, where it was cut into small fragments. These fragments were put into an inoculating trocar designed for the purpose of transplanting tumor fragments. Approximately 0.5 Gm. of tissue was used for each inoculating dose. The experimental chickens were 2 months old at the time of inoculation. Four received the inoculum in the breast muscle and four in the peritoneal cavity. In no instance was there indication of growth of the implants and the experiment was discontinued after 8½ weeks.

Such an experimental period may be regarded as short in comparison with the 12- to 18-month period allowed in the work of some investigators studying the question of transmissibility of lymphocytoma. The nature of the experiment had a definite bearing upon this question. In this case an attempt was being made to transplant the tumor with viable cells. If growth were to take place, it should have become manifest well within the period of 8½ weeks allowed. In the case of the transplantable lymphoid tumor developed from case 452, growths of implants of viable cells were usually apparent within two or three weeks, and if growth did not occur within that time, the birds remained negative.

Case 452.—Lymphoid Tumor: The transmissible lymphoid tumor was derived from this case. The anatomic pathology of the case was similar to that of lymphocytoma. The term lymphocytoma is not applied to this case, however, because of the transmissible character of the neoplasm, a feature which can not be readily demonstrated in cases of lymphocytoma. Future work may be expected to clarify this situation.

A detailed description of case 452 is given in another report.[1] A brief résumé follows:

The chicken was noted ill at the age of 146 days when a marked leucocytosis was evident. Death occurred at the age of 205 days. At necropsy the liver, spleen, bursa of Fabricius and bone marrow contained macroscopic foci of gray-white tumor tissue. Upon microscopic examination the ovary was found to be infiltrated. The type cell of the neoplasia was a large lymphoid cell, principally extravascular in location, although a few such cells were apparent in the sinusoids of the liver.

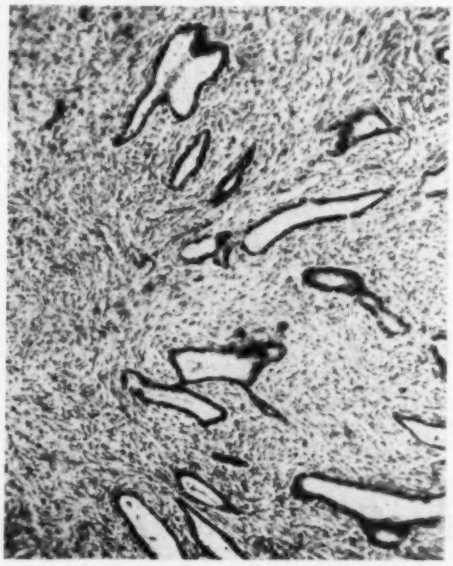

Fig. 3. Carcinosarcoma (case 922). Section taken from a tumor nodule of the mesentery. The tumor is composed of two elements: irregular acini of epithelial cells and fibroblasts (x 60).

Portions of the liver involved with neoplastic tissue were used as implants for experimental chickens. Blood also was obtained and used as an inoculum for experimental chickens. The lymphoid tumor was successfully transplanted with reproduction of the same disease. The detailed results of serial transmission are given in another report.[1]

Discussion and Summary

Neoplastic disease was unusually common in the flock studied. The 13 cases of tumor observed in 48 chickens represent

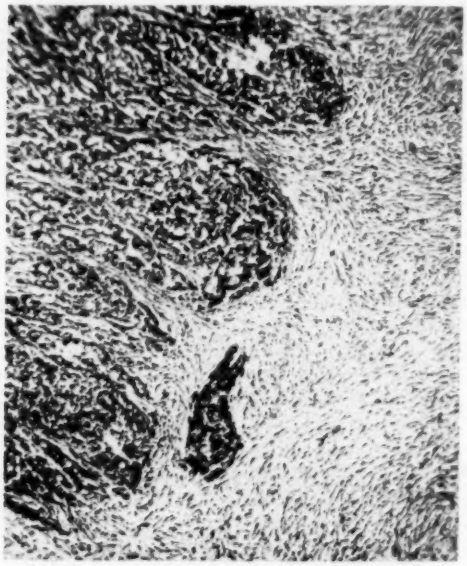

Fig. 4. Carcinosarcoma (case 922). A tumor nodule different from that illustrated in figure 3. Here the epithelial elements form solid masses of very small acini (x 60).

an incidence of 27 per cent. The incidence in females was 57 per cent (12 cases among 21 birds). Only one case was observed in a male chicken. It must be pointed out that an exact comparison can not be made between the sexes, as the cockerels were killed at a much earlier age than the females and the numbers under observation were small.

A variety of neoplastic disease was found. The most common type was that involving lymphoid cells (lymphocytoma, five cases). There were two cases each of fowl leucosis, fibrosarcoma and carcinoma. Neurogenic sarcoma (one case) has not heretofore been described in the chicken. Fowl paralysis (neurolymphomatosis) did not occur in the flock.

The two cases of fowl leucosis were reproduced in experimental chickens. Two cases of neoplasia of the lymphoid cell system furnished material suitable for attempts at experimental transmission. In one case the attempts were successful. The transmissible diseases reproduced in experimental birds were essentially similar to the diseases in the chickens from which the material was originally obtained.

The results obtained would seem to indicate that different agents were responsible for the two types of transmissible neoplastic disease found in the flock.

The Experimental Use of Organic Sulfur Compounds for the Prevention of Cecal Coccidiosis in Chickens*

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COCCIDIOSIS IN POULTRY is a widespread and devastating disease caused by a protozoan of the genus Eimeria. Several species of coccidia infect poultry, but only two are feared. One of these, *Eimeria necatrix*, invades the endothelial cells of the small intestine, while the other, *Eimeria tenella*, invades the cells lining the cecal pouches. The present study was confined entirely to *E. tenella*, which causes cecal coccidiosis.

There is no known drug that will kill the protozoan causing cecal coccidiosis after it has once penetrated the cells lining the digestive tract. Many drugs have been suggested for the prevention of coccidiosis, but sanitation is the method most generally used to prevent losses from this disease.

In 1936, Herrick and Holmes[1] proposed the use of elemental sulfur as a preventive. In 1937, these investigators[2] called attention to several of the injurious effects of sulfur feeding and reported that several days of continuous feeding generally were required for sulfur to exhibit its full value as a preventive and that the protective value of sulfur disappeared within a few days following discontinuance of the drug in the feed. This lag in the full effectiveness of sulfur as a preventive for coccidiosis was considered to be of importance especially in cases in which sulfur was not being fed and coccidiosis suddenly appeared in a flock. As a result, a search was made to find a form of sulfur that would be effective immediately. A colloidal sulfur produced by a local firm was tried, but it was found to be effective only under certain conditions and did not completely solve the problem. Many inorganic sulfur compounds were tried without a great deal of success.

One of the authors wrote to Dr. W. H. Tisdale, manager of the Pest Control Research Section, Grasselli Chemicals Department, E. I. du Pont de Nemours & Company, and asked if he could supply a sulfur compound with certain specified physical and chemical properties. Dr. Tisdale supplied this compound, which proved to be successful as a preventive for coccidiosis. The du Pont Company then established an industrial fellowship at the University of Wisconsin to study the effectiveness of this product and of several other organic sulfur compounds that they had found effective for other purposes. As a result, eleven sulfur compounds were investigated in a preliminary way to determine their value as preventives of coccidiosis.

MATERIALS AND METHODS

The chickens used were Single Comb White Leghorns, Barred Plymouth Rocks and Rhode Island Reds in the approximate proportions of 80, 10 and 10 per cent, respectively. These chickens were furnished by the Department of Poultry Husbandry of the University of Wisconsin.

Unless otherwise stated, the chicks were removed from the incubator to a building which housed only uninfected chickens and where great precautions were taken to prevent accidental infection. When the chicks were placed on experiment they were removed to a building where they were kept in cages and under conditions described by Herrick, Ott, and Holmes.[3] At no time was there any accidental infection among the chickens. The feed used was composed of ground yellow corn, wheat bran, stand-

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[1]Herrick, C. A., and Holmes, C. E.: Effects of sulfur on coccidiosis in chickens. Vet. Med., xxxi (1936), pp. 390-391.

[2]Herrick, C. A., and Holmes, C. E.: Coccidiosis in chickens. Spec. Bul. Wisconsin Agr. Exp. Sta. (1937).

[3]Herrick, C. A., Ott, G. L., and Holmes, C. E.: The chicken as a carrier of the oöcysts of the coccidia *Eimeria tenella*. Poultry Sci., xv (1936), pp. 322-325.

TABLE I—Toxic Effect of Eleven Organic Sulfur Compounds on Chickens and Their Effectiveness in Preventing the Development of Coccidia

Compound	Dose per Chick	No. Chicks	Weight of Chicks (Gm.)	Toxicity	Protection from Coccidiosis
I	1 cc.	2	1,210	0	Complete
	2 cc.	2	1,200	0	Complete
	3 cc.	2	1,270	0	Complete
	4 cc.	2	1,180	0	Complete
II	1 cc.	2	1,300	0	Complete
	2 cc.	2	1,240	0	Complete
	3 cc.	2	1,410	0	Complete
	4 cc.	2	1,520	0	Complete
III	1 Gm.	2	1,500	0	0
	2 Gm.	2	1,200	0	0
	3 Gm.	2	1,250	0	0
	4 Gm.	2	1,250	0	0
IV	1 cc.	2	1,100	0	Complete
	2 cc.	2	1,130	0	Complete
	3 cc.	2	1,200	0	Complete
	4 cc.	2	1,220	0	Complete
V	1 Gm.	2	1,400	0	Complete
	2 Gm.	2	1,300	0	Complete
	3 Gm.	2	1,450	0	Complete
	4 Gm.	2	1,400	0	Complete
VI	0.5 Gm.	2	1,200	0	Slight
	1 Gm.	2	1,100	Killed	
	2 Gm.	2	1,280	Killed	
	3 Gm.	2	1,340	Killed	
	4 Gm.	2	1,850	Killed	
VII	1 Gm.	2	1,650	0	0
	2 Gm.	2	1,290	0	0
	3 Gm.	2	1,970	0	0
	4 Gm.	2	1,900	+	0
VIII	1 Gm.	2	1,710	0	0
	2 Gm.	2	1,400	0	0
	3 Gm.	2	1,180	0	Very slight
	4 Gm.	2	1,400	0	Slight
IX	1 Gm.	1	1,580	0	0
	2 Gm.	1	1,500	0	Very slight
	3 Gm.	1	1,610	+	
	4 Gm.	1	1,550	++	
X	1 Gm.	1	1,005	0	0
	2 Gm.	1	900	0	0
	3 Gm.	1	1,100	+	Very slight
	4 Gm.	1	1,000	++	Slight
XI	1 Gm.	2	1,400	0	0
	2 Gm.	2	1,450	0	0
	3 Gm.	2	1,450	0	0
	4 Gm.	2	1,825	0	0

+One or very few lesions. ++Slight lesions.

ard wheat middlings, ground oats, alfalfa meal, fish meal, meat scrap, soybean oil meal, cod liver oil, whole yellow corn, whole wheat and whole oats. The chicks were hopper fed and supplied with running water at all times. They were given both the sulfur compounds and the oöcysts by means of graduated pipettes. The chickens received no preparation before treatment; they were not starved and both the compounds and oöcysts were administered into

full crops. Due to the clumping of the oöcysts even when great precautions were taken to grind up the cecal contents in preparing the oöcysts for sporulation, it was difficult to secure accurate counts. An effort was made to administer 200,000 sporulated coccidia oöcysts at each feeding.

The surviving chickens were autopsied by the ninth day following infection and the severity of the lesions noted. If one or very few lesions were visible, the chickens were marked as + ; if lesions were slight, ++ ; if many lesions were present, with considerable blood, +++ ; and if the lesions were severe, with large amounts of blood in the pouches, they were marked ++++.

In order not to be influenced in our judgment regarding the severity of the lesions of the various chickens, all birds to be killed during one day regardless of treatment were examined at one time and those of like lesions placed together. It was not until after our records were complete that the chickens were identified by their wing numbers.

The sulfur compounds studied were:

No. *Chemical Name*

I. Dodecyl thiocyanate

II. "Lorol" thiocyanate—a commercial mixture of straight chain aliphatic thiocyanates derived from a cut of the alcohols obtained by hydrogenation of coconut oil, and having approximately the following composition:

Octyl thiocyanate	12%
Decyl thiocyanate	8%
Dodecyl thiocyanate	56%
Tetra decyl thiocyanate	24%

III. Mercaptobenzothiazole

IV. Tetraethylthiuram monosulfide

V. A water emulsion containing 25% tetraethylthiuram monosulfide and 25% coconut oil as the internal phase.

VI. Tetramethylthiuram monosulfide

VII. Tetramethylthiuram disulfide

VIII. Ferric dimethyldithiocarbamate

IX. 3, 7 Dithia 1,5-diazabicyclo-(3.3.1) nonane

X. Contains 50% IX with inert dispersing agents

XI. Phenothiazine

Due to the length of the chemical names of the compounds used, they will be referred to by their respective numbers only.

EXPERIMENTAL PROCEDURE

Preliminary Tests.—Each of the eleven compounds was administered to chickens which were given sporulated coccidia oöcysts within two hours. The results of this experiment are shown in table I.

Compounds I, II, IV and V were relatively nontoxic to chickens and gave protection from coccidiosis. These compounds were, therefore, reserved for further study. The seven less promising compounds were not studied further. From a study of the list of compounds it is seen that compound I is pure dodecyl thiocyanate, while II is a mixture of thiocyanates. Since compound I was more expensive and in detailed studies seemed to have no advantage over compound II, the experiments on this compound are not included. It is further seen that compound V is formed from IV by suspending 25 per cent tetraethylthiuram monosulfide in coconut oil. Although these compounds are basically the same, both were retained for further study because of the great difference in their physical characteristics.

Experiments on Single Doses of Compounds II, IV and V.—Compound II is an oily liquid with a characteristic and penetrating odor. It is immiscible with water, but may be readily dissolved in many oils. Studies on this compound showed that in single doses it was well tolerated; no toxic symptoms were produced until .005 cc. per gram of body weight was given and then only in chickens weighing less than 300 Gm. (See table II.)

Doses of .006 cc. per gram were toxic to nearly adult chickens. Doses of .01 cc. per gram and above produced increasingly more serious toxic symptoms and a dose of .05 cc. per gram was 100 per cent fatal to young chickens.

To test the efficacy of compound II as a preventive for cecal coccidiosis, doses of from .001 to .00001 cc. per gram of body weight were given to chickens of various weights and for varying periods before the oöcysts were fed. The results of these experiments are shown in table III.

When the compound was given 17 hours before the oöcysts were fed, it required only .0004 cc. per gram to prevent all symptoms of coccidiosis. These chickens were autopsied on the ninth day following infection and none showed macroscopic lesions of coccidia in the cecal pouches. When .0003 cc. per gram was given, slight lesions resulted. Doses below .0003 cc. per gram gave

TABLE II—Results of Experiment Conducted to Determine the Minimal Lethal Dose of Compound II for Chickens

Dose (cc.) per Gram Weight	Av. Weight (Gm.)	No. of Chicks	Toxic Symptoms	Per Cent Mortality	Remarks
Controls	912	4	0	0	
.003	300	6	0	0	
.003	1,300	3	0	0	
.004	320	6	0	0	
.005	290	5	++++	60	Death in 24 hrs.
.005	842	10	0	0	
.005	1,000	3	0	0	
.006	320	6	++++	50	Death in 24 hrs.
.006	804	10	++	0	
.006	1,010	2	0	0	
.01	600	2	++	0	
.02	380	2	+++	0	
.03	415	4	++++	0	
.05	480	2	++++	100	Death in 12 hrs.

++Slight lesions.
+++Many lesions, with considerable amount of blood.
++++Severe lesions, with large amount of blood.

erratic results and when doses of .0001 cc. per gram were given, severe coccidiosis resulted, but in no case did any of the chickens, treated 17 hours before being infected, die whereas 56 per cent of the untreated chickens died of coccidiosis.

Chickens given the compound only 30 minutes before receiving the coccidia oöcysts were not afforded as much protection as those given the compound 17 hours previous to infection. Table III shows that even when .0004 cc. per gram was given to young chickens (100 Gm.), severe coccidiosis resulted and 43 per cent of the chickens died. When as little as .0003 or .0002 cc. per gram was given, 57 per cent of the treated chicks died of coccidiosis. These chickens apparently were given some protection, for 80 per cent of the untreated controls of the same weight died of coccidiosis.

It is evident from a study of the data given in tables II and III that compound II, when given in tolerated doses, did give protection from coccidiosis even in single doses and in chickens on full feed. The ratio of toxic and protective doses of the compound is shown in table III to be 1:10.

Compound IV is an oily liquid with a distinctive and penetrating odor that remains for long periods. It may be mixed readily and emulsified in most oils, but is immiscible with water. It was found to be nontoxic to chicks when given in doses of .006 cc. per gram. It was, however, definitely toxic and killed 32 per cent of the animals when given in a dosage of .008 cc. per gram. (See table IV.) The surviving animals recovered rapidly and seemingly suffered no lasting effects from the compound. Those animals which received more than .008 cc. per gram succumbed in a majority of cases and the survivors recovered slowly.

As a preventive for cecal coccidiosis, compound IV was effective in doses of .0004 cc. per gram if given six hours before the oöcysts were fed (table V). Doses less than .0004 cc. per gram gave less consistent results and in some cases severe coccidia lesions developed, but serious coccidiosis or mortality did not result even when sufficient oöcysts were fed to kill 78 per cent of the untreated chickens. When the compound was given 30 minutes before the oöcysts were fed, the results were less uniform, and when .0002 cc. was given, 50 per cent of the birds died of coccidiosis. A dose of .0003 cc. per gram allowed severe coccidiosis to develop, but none of the animals died.

When the lighter doses of compound IV were given, macroscopic lesions were absent many times, but large numbers of oöcysts developed. Experiments are now in progress to determine if those which develop in such cases become attenuated or if they become increasingly resistant after several generations of development under such conditions.

When compound IV is compared with II (see tables III and V), it is apparent that both have about the same value as a preventive, but IV was less toxic to chickens; the ratio between the protective and nontoxic dose was found to be 1:20 as compared with 1:10 for compound II.

Compound V is a yellowish emulsion dispersible in water in all proportions. It has the characteristic unpleasant taste of No. IV. As has been stated, compound V is a 25 per cent emulsion of IV. It was found, however, that it was relatively more toxic than IV (table VI), possibly due to the presence of emulsifying agents. Its toxicity was clear cut. It was seemingly nontoxic in doses of .009 cc. per gram of body weight, but .01 cc. was toxic and killed 12.5 per cent of the treated animals within 24 hours. Doses of .03 cc. per gram were fatal in all cases and the chickens were dead in ten hours or less.

As a preventive for cecal coccidiosis, V was found to be effective, but only in relatively large doses—doses near the toxic limit. Doses of .008 cc. per gram were found to be protective when given either 30 minutes or 6 hours previous to administering the oöcysts (table VII), but doses

TABLE III—Results of Experiment Conducted to Determine Minimal Protective Dose of Compound II Against Cecal Coccidiosis

Dose (cc.) per Gram Weight	Time Before Oöcysts Fed After Chemical	Av. Weight (Gm.)	No. of Chicks	Coccidia Symptoms	Macroscopic Lesions	Per Cent Mortality
.001	17 hrs.	730	10	0	0	0
.0009	17 hrs.	800	4	0	0	0
.0008	17 hrs.	760	4	0	0	0
.0007	17 hrs.	700	4	0	0	0
.0005	17 hrs.	750	4	0	0	0
.0004	17 hrs.	750	4	0	0	0
.0004	17 hrs.	141	10	0	0	0
.0004	30 min.	100	7	0—+++	0—++++	43
.0004	30 min.	590	7	0	0—+++	0
.0003	17 hrs.	700	4	0	0—+	0
.0003	17 hrs.	153	10	0	0—++	0
.0003	30 min.	100	7	0—+++	+—++++	57
.0003	30 min.	600	9	0—+	0—+++	0
.0002	17 hrs.	650	4	0—+	++	0
.0002	17 hrs.	158	10	0	0—+++	0
.0002	30 min.	93	7	+—+++	++++	57
.0002	30 min.	650	8	0—++	++—++++	0
.0001	17 hrs.	720	7	++	+++	0
.0001	17 hrs.	144	9	0	0—++	0
.0001	30 min.	550	9	0—+++	+++	0
.0001	30 min.	90	7	0—+	0—++++	43
.00009	17 hrs.	735	4	+++	+++	0
.00008	17 hrs.	760	4	+++	+++	0
.00007	17 hrs.	700	4	+++	+++	0
.00006	17 hrs.	710	4	+++	+++	0
.00004	17 hrs.	750	4	+++	+++	0
.00003	17 hrs.	750	4	+++	++++	0
.00001	17 hrs.	730	4	++++	++++	0
Controls		100	5	++++	++++	80
Controls		750	17	+++	++++	56

+One or very few lesions.
++Slight lesions.
+++Many lesions, with considerable amount of blood.
++++Severe lesions, with large amount of blood.

TABLE IV—Results of Experiment Conducted to Determine Minimal Lethal Dose of Compound IV for Chickens

Dose (cc.) per Gram Weight	Av. Weight (Gm.)	No. of Chicks	Toxic Symptoms	Per Cent Mortality	Remarks
Controls	317	6	0	0	
.004	340	6	0	0	No noticeable effect
.005	337	7	0	0	No noticeable effect
.006	345	7	0	0	No noticeable effect
.008	331	7	+	32	Recovery rapid
.0095	382	9	++++	66	Dead in 12 hrs.
.017	356	8	++++	100	Dead in 8 hrs.

+One or very few lesions.
++++Severe lesions, with large amount of blood.

TABLE V—Results of Experiment Conducted to Determine Minimal Protective Dose of Compound IV Against Cecal Coccidiosis

Dose (cc.) per Gram Weight	Time Before Oöcysts Fed After Chemical	Av. Weight (Gm.)	No. of Chicks	Coccidia Symptoms	Macroscopic Lesions	Per Cent Mortality
.0008	30 min.	240	6	0	0	0
.0004	30 min.	235	6	0	0	0
.0003	30 min.	290	7	+	+++	0
.0003	6 hrs.	320	8	0	0	0
.0003	6 hrs.	151	8	0	0	0
.0002	30 min.	250	6	+++	++—++++	50
.0002	6 hrs.	415	12	0	0—+	0
.0002	6 hrs.	310	6	0	0—++	0
.0001	6 hrs.	410	12	0	+	0
.00004	6 hrs.	366	6	0	++	0
.00004	30 min.	250	6	+++	++—++++	33
Controls		250	9	++++	++++	78

+One or very few lesions.
++Slight lesions.
+++Many lesions, with considerable amount of blood.
++++Severe lesions, with large amount of blood.

TABLE VI—Results of Experiment Conducted to Determine Minimal Lethal Dose of Compound V for Chickens

Dose (cc.) per Gram	Av. Weight (Gm.)	No. of Chicks	Toxic Symptoms	Per Cent Mortality	Remarks
.003	185	6	0	0	
.005	200	12	0	0	
.007	320	6	0	0	
.008	254	5	0	0	
.009	254	5	0	0	Death:
.01	360	8	++	12.5	Within 24 hrs.
.015	450	4	++	25.0	Within 24 hrs.
.02	400	8	++++	87.5	Within 24 hrs.
.03	500	2	++++	100	Within 10 hrs.
.05	400	2	++++	100	Within 8 hrs.

++Slight lesions.
++++Severe lesions, with large amount of blood.

of .01 cc. proved to be fatal in some cases. The ratio between the preventive dose and the nontoxic dose was only 1:1.12.

Continuous Feeding of Compounds II, IV and V.—In some preliminary experiments not reported here it was found that the experimental compounds did not remain effective in preventing coccidiosis for more than three days. It is obvious, therefore, that the respective compounds would have to be almost constantly present in the digestive tract to be effective in preventing the disease.

To test the effect of the various compounds on the rate of growth and the resistance of the chickens to coccidiosis, the various compounds were fed over varying periods.

Compounds II and IV were fed to eight groups of chickens when they were 24 days old and until the termination of the experiment, as shown in table VIII. After the chickens had received the respective compounds for eleven days, the chickens in one half of the groups were each given 200,000 coccidia oöcysts. The remaining five groups continued to receive treatment until they were 64 days old.

It is obvious that compounds II and IV did not afford any protection from coccidiosis. In fact, the treated groups suffered a much higher mortality than did the control group. Why this was so is not known. It is suggested that all chickens picked up coccidia oöcysts more or less constantly and developed in the controls, which caused the controls to develop a resistance, whereas the treated groups were protected from the very light infections and did not develop resistance. The amount of compounds received by the chickens was obviously not sufficient to protect against large numbers of coccidia.

Except for the group receiving 0.1 per cent of compound II, the treated groups made greater gains than did the control groups. This may have been due to mild coccidiosis suffered by the control group.

Since eleven days of continuous feeding did not prove sufficient to protect against coccidiosis, compound II was fed continuously for 8 weeks to test its efficacy as a preventive and for 20 weeks to test its effect on the rate of growth. The chicks on this experiment were kept in electrically heated brooder houses (4'x6') to which were attached sand yards (4'x8'). The

TABLE VII—Results of Experiment Conducted to Determine Minimal Protective Dose of Compound V Against Cecal Coccidiosis

Dose (cc.) per Gram Weight	Time Before Oöcysts Fed After Chemical	Av. Weight (Gm.)	No. of Chicks	Coccidia Symptoms	Macroscopic Lesions	Per Cent Mortality
.008	30 min.	200	6	0	0	0
.008	6 hrs.	250	6	0	0	0
.005	6 hrs.	145	6	0	+—+++	0
.005	30 min.	180	9	0	0—+++	0
.004	6 hrs.	157	7	0	+—+++	0
.004	30 min.	150	9	0	+—+++	0
.004	30 min.	282	6	0	0—+	0
.003	30 min.	320	7	0	0—++	0
.002	30 min.	290	14	+—+++	0—+++	22
.0004	30 min.	490	5	++	+++	20
.0004	24 hrs.	470	5	+++	++++	60
.0003	30 min.	365	5	+++	++++	40
.0003	24 hrs.	445	4	+++	++++	25
.0002	30 min.	443	5	+++	++++	40
.0002	24 hrs.	520	5	++++	++++	60
Controls		200	12	++++	++++	75

+One or very few lesions.
++Slight lesions.
+++Many lesions, with considerable amount of blood.
++++Severe lesions, with large amount of blood.of blood.

TABLE VIII—Effect of Compounds II and IV on Gains in Weight and on Resistance to Coccidia; Feeding Was Begun When Chicks Were 24 Days Old; Chicks Infected After Receiving Respective Amounts of Compounds for 11 Days

	Compound II				Compound IV				Controls	
Amt. of cmpd.*	.05	.05	.1	.1	.05	.05	.1	.1	†	
No. of chicks	35	28	34	32	34	30	36	29	32	35
Age of Chicks in Days	Weight in Grams									
1	40.0	41.4	39.1	39.0	38.8	39.4	33.3	40.6	39.7	40.3
24	172.5	168.0	171.0	160.0	146.8	152.4	175.1	168.3	167.4	146.0
35	298.2	265.1	262.2	228.0	247.0	252.8	251.5	251.4	283.3	265.0
64		667.7		557.5		694.8		639.7		622.7
Mortality %		57.1		35.3		23.5		47.5		8.6

*Percentage of ration.
†Given approximately 200,000 oöcysts of *E. tenella* when 24 days old.

compound was administered as shown in table IX.

During the first two weeks all groups made comparable gains in weight, but shortly thereafter group 6 (receiving 0.18 per cent of compound II) began to fall behind in gains. They ate but little and many died. When 4 weeks old, it was obvious that all or nearly all would die if continued on experiment. They were, therefore, changed to the control ration, and they improved rapidly. The group receiving 0.15 per cent showed the same general symptoms of weakness and loss of appetite, but improved when the amount of the compound was reduced. The one group received 0.12 per cent throughout the entire 20 weeks, but made smaller gains than did the other groups. From the data shown in table IX, it is obvious that the chicks, particularly those receiving 0.12 per cent, were getting the maximum tolerated amount of the compound.

When the chickens receiving compound II were 8 weeks old, the males from all groups were removed and each given 200,-

TABLE IX—Average Gains in Weight of Chickens Fed Varying Amounts of Compound II for 20 Weeks

No. of Chicks	Amounts of Compound II	Average Weight in Grams at							Per Cent Mortality
		Start	2 Weeks	4 Weeks	8 Weeks*	12 Weeks	16 Weeks	20 Weeks	
50	Basal	39.3	108.4	219.4	533.8	789.0	1177.5	1491.0	7.4
50	0.09%	39.1	101.5	176.9	431.8	697.0	1092.0	1336.0	5.5
50	0.12%	39.1	96.5	162.4	418.1	610.0	994.1	1227.0	10.9
50	Basal	38.6	100.4	198.1	499.8	796.0	1204.5	1495.0	7.3
50	0.15% first 4 weeks and 0.03% 4-20 weeks	38.7	110.4	175.6	458.1	705.4	1113.3	1330.5	7.1
50	0.18% first 4 weeks and basal only 4-20 weeks	39.1	100.9	130.2	418.7	715.9	1140.0	1386.0	14.3

*Pullets only; cockerels removed at 8 weeks of age.

000 oöcysts. The chickens receiving compound II were not protected from coccidiosis; the mortality was as high as in the control group.

Thus, it seems that it was not possible to give compound II continuously in the feed in such quantities that it would protect from massive infections of coccidia and not materially injure the chickens.

When the birds in group 6 (which was started on 0.18 per cent of compound II) were 20 weeks old, they were given a ration containing 0.1 per cent of compound IV to test its effect on the egg production of the pullets. It is interesting that as long as this ration was given (six weeks), the pullets layed very few eggs. Within two weeks after the compound was removed from the ration, the pullets were in production nearly equal to that of the controls. No explanation can be given for its effect on egg production.

Compound V was fed continuously in the feed, but with unsatisfactory results. The crops soon began to fill up and became hard. The contents had a gummy texture and did not seem to pass into the gizzard. It is suggested that the dispersing medium may have increased the toxic principle of this compound and caused a paralytic action as is recorded for this compound on certain arthropods.

The Use of Compound IV to Increase Resistance.—As previously stated, it often was noted in chickens receiving compound IV that although no symptoms were noted and macroscopic coccidial lesions were slight or even absent, many oöcysts might be present. This suggested the possibility that one might so adjust the dosage of compound and the number of oöcysts fed that resistance might be developed by a light infection of coccidiosis without seriously injuring the chicken. Experiments were carried out to test this hypothesis. In these experiments a group of chickens were given a certain amount of the compound and numbers of oöcysts at the same time. At a later time the chicks were given a second feeding of oöcysts, but none of the compound was given. The results of these experiments are shown in table X.

It is evident that losses from the first infection were prevented by the compound, yet considerable resistance was developed to the second. In the case of the 3-day-old group it is suggested that too much of the compound was given. This so completely protected the chickens from the first infection that little resistance was acquired to protect from the second infection. These experiments are too few to give conclusive evidence, but if the action of the compound is to reduce the productivity or virulence

TABLE X—Use of Compound IV to Develop Resistance to Coccidiosis in Chickens; a Subprotective Dose of Compound IV Was Given to Control First Infection; No Compound Was Given at Time of Second Infection

No. of Chicks	Age (Days)	Dose per Gm.	Mortality Per Cent	Time (Days) After 1st Inf.	Lesions: (Per Cent)					Mortality Per Cent
					0	+	++	+++	++++	
20	42	.00026	0	65	50.0	10.0	5.0	20.0	0	15
6*	42	.....	83	....	.....	.....	.....	.....	....	....
8†	107	.....	....	....	.....	.....	.....	.....	50	50
13	7	.0009	0	22	15.0	23.0	62.0	0	0	0
4*	7	.....	100	....	.....	.....	.....	.....	....	....
6†	29	.....	....	....	.....	.....	.....	.....	50	50
21	3	.0016	0	68	9.0	28.0	23.0	9.0	0	31
5*	3	.....	100	....	.....	.....	.....	.....	....	....
5†	71	.....	....	....	.....	.....	.....	.....	....	100

*Controls, first infection.
†Controls, second infection.
+One or very few lesions.
++Slight lesions.
+++Many lesions, with considerable amount of blood.
++++Severe lesions, with large amount of blood.

TABLE XI—Effect of Compound II on the Viability of E. Tenella Oöcysts

Time Oöcysts in Comp. II	Weight (Gm.) of Chicks	No. of Chicks	Oöcysts Produced	Per Cent Mortality
5 days	450	6	Abundant	83
Controls	460	3	Abundant	66
8 days	460	4	Abundant	25
Controls	450	3	—	100
16 days	440	4	Few	0
Controls	460	3	—	100
26 days	475	5	None	0
Controls	480	3	—	100

of the oöcysts and result in an attenuated case of coccidiosis followed by resistance or immunity, such a method might be of value in combating coccidiosis in the field. Experiments along this line are being continued with the view to developing a practical control.

The Effect of Compound II on the Viability of the Oöcysts.—Compound II was very effective as a preventive and when no gross lesions were apparent in the ceca, no oöcysts developed. It was, therefore, decided to test its effect on the viability of the sporulated oöcysts.

Washed oöcysts were placed in a quantity of unadulterated compound II and tested after periods of 5, 8, 16 and 26 days. When it was desired to test them, they were washed many times in olive oil to remove as much of the compound as possible. They were then administered to nonimmune chickens and compared with chickens given like numbers of untreated oöcysts. It was found that after 16 days their viability was somewhat reduced and they evidently were killed after a period of 26 days (table XI). It is evident that this compound would be of no practical value if applied to oöcysts under range conditions.

DISCUSSION

Ten synthetic nitrogen-sulfur compounds, which previously proved effective against certain other parasitic organisms, were tested against cecal coccidia in chickens and three of them found to give protection. One of these compounds, tetraethylthiuram monosulfide, which was used in unadulterated form (No. IV) and in coconut oil emulsion (No. V) was, in the unadulterated form, seemingly the most satisfactory of the chemicals used, for it was the least toxic to chickens. "Lorol" thiocyanate (No. II) and tetraethylthiuram monosulfide were both more effective if given several hours before the oöcysts were fed, but were effective in larger doses if given at the same time the oöcysts were fed. It was evident that young chickens required a relatively larger dose for protection.

Single effective doses of these compounds seemed to be rather well tolerated. Continuous feeding was found to be impractical under the conditions of these experiments due to lack of coccidiosis control with both products and to injury to the chickens, which was more pronounced with "Lorol" thiocyanate. Further research, however, may reveal a satisfactory method of administration through the feed. The chickens receiving continuous doses of "Lorol" thiocyanate and tetraethylthiuram monosulfide prove less resistant to massive infections of coccidia than did the controls. This may indicate that the treated chickens were being protected against light infections of coccidia that produced resistance in the controls and, therefore, made the controls more resistant to the massive infections. There is a question as to whether such massive infections would often be encountered on poultry farms. Further research may show that much smaller amounts of these compounds will protect chickens from coccidial infections acquired on most poultry farms. Research along this line is indicated.

The use of tetraethylthiuram monosulfide (No. IV) to regulate the severity of the initial infection by coccidia and thereby produce resistance to subsequent infections is of considerable interest. Further research on this phase of the subject is needed, in order to develop a practical

method of administering the chemical and the coccidia to insure coccidiosis control.

SUMMARY

1) "Lorol" thiocyanate and tetraethylthiuram monosulfide, when administered in single doses, were effective in preventing the development of cecal coccidiosis in chickens.

2) Continuous feeding of these chemicals under the conditions of these experiments proved unsuccessful. However, further research might disclose a satisfactory means of administration in the feed.

3) Tetraethylthiuram monosulfide, when administered in suitable dosages at the time of the administration of coccidia oöcysts, offers promise for developing resistance to coccidiosis in chickens. Further research is needed to develop a practical method of application of the chemical and the oöcysts to produce resistance.

Organization of the Research Council, American Veterinary Medical Association

The Research Council of the American Veterinary Medical Association was formed at the seventy-eighth annual meeting of the Association* last summer for a twofold purpose: (1) to review, with respect to scientific merit, the manuscripts offered for publication in the American Journal of Veterinary Research and (2) to plan and supervise a program for encouraging veterinary research. This council is composed of 14 members chosen to represent the various fields of veterinary science. Each is responsible for passing on those articles submitted for publication which fall within the scope of his activity. The organization of the Council is as follows:

E. T. Hallman, *Chairman*, Michigan State College, East Lansing, Mich.

R. A. Kelser, *Vice-Chairman*, Office of the Surgeon General, U. S. Army, Washington, D. C.

H. H. Dukes, *Secretary*, New York State Veterinary College, Ithaca, N. Y.

Anatomy and Histology: H. L. Foust, Iowa State College, Ames, Iowa.

Bacteriology (Immunology and Biologic Therapy): Edward Records, University of Nevada, Reno, Nev.

Biochemistry and Animal Nutrition: George H. Hart, University of California, Davis, Calif.

Large Animal Medicine: James Farquharson, Colorado State College, Fort Collins, Colo.

Large Animal Surgery: W. F. Guard, The Ohio State University, Columbus, Ohio.

Parasitology: E. W. Price, U. S. Bureau of Animal Industry, Washington, D. C.

Pathology: E. T. Hallman, *Ibid.*

Physiology and Pharmacology: H. H. Dukes, *Ibid.*

Small Animal Medicine: M. L. Morris, New Brunswick, N. J.

Small Animal Surgery: C. F. Schlotthauer, Mayo Foundation, Rochester, Minn.

Veterinary Hygiene: W. E. Cotton, Alabama Polytechnic Institute, Auburn, Ala.

Virus Diseases: R. A. Kelser, *Ibid.*

X-Ray: M. A. Emmerson, University of Pennsylvania, Philadelphia, Pa.

Member-at-Large: E. A. Watson, Animal Disease Research Institute, Hull, Que.

Editorial Procedure

At a meeting in Chicago, December 1, 1941, the Council adopted the following rule of procedure with respect to articles offered for publication:

> Scientific papers found by the editor of the American Journal of Veterinary Research to be appropriate for publication shall be referred to the member of the Council having the major interest in the subject. The member will return the paper to the editor within two weeks, together with his recommendation for acceptance or rejection. If rejection is recommended, the reasons therefor are to be stated. The editor will then notify the author as to whether the paper has been accepted or rejected. If rejected, and the author requests that rejection be reconsidered, the paper will be returned to the chairman of the Council for action of the whole body.

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*Proceedings, seventy-eighth annual meeting, American Veterinary Medical Association. J.A.V.M.A., xcix (Oct. 1941), pp. 349-352.